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Connecting the Dots: Understanding Migration in the Context of Other Periods of the Annual Cycle

Kristina Lee Paxton
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The University of Southern Mississippi

CONNECTING THE DOTS: UNDERSTANDING MIGRATION IN THE CONTEXT
OF OTHER PERIODS OF THE ANNUAL CYCLE

by

Kristina Lee Paxton

Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

December 2013

ABSTRACT

CONNECTING THE DOTS: UNDERSTANDING MIGRATION IN THE CONTEXT OF OTHER PERIODS OF THE ANNUAL CYCLE

by Kristina Lee Paxton

December 2013

Migration is a fundamental characteristic of the life history of many organisms. Large-scale seasonal movements expose migratory species to an array of differing environmental conditions such that the ecology, behavior, and life history strategies of migratory species must balance selection pressures associated with each phase of the annual cycle. Therefore, scientists increasingly realize that to understand the ecology of a migratory species in any one phase of the annual cycle requires an understanding of how other phases may interact with and influence the period of interest. That said, following individuals throughout the year poses a serious challenge.

The focus of my dissertation research was to examine factors important to migration in the context of other periods of the annual cycle. From a broad-scale perspective, I examined how global climatic cycles such as El Niño Southern Oscillation in geographically different regions of the world carry-over to impact the migratory success of numerous intercontinental migratory bird species. I directly associated climatic variability experienced at over-winter areas with factors important to the success of migration, namely timing and condition, providing strong evidence that not only are migratory birds during spring migration influenced by events occurring during the previous phase of their annual cycle, but where they over-winter determines how vulnerable they are to global climatic cycles. From an individual-scale perspective, I

examined (1) how conditions prior to the onset of migration carry-over to affect migration the success of black-and-white warblers (*Mniotilta varia*), and (2) how a warbler's migration stopover strategy interacts with other periods of the annual cycle. I found that the quality of a bird's over-winter habitat strongly influenced the timing of migration with cascading impacts on a warbler's migration strategy during stopover. However, the distance remaining to a bird's final breeding area destination also played a strong role in the strategy a bird utilized at a stopover site. Last, I integrated information from stable isotopes and genetic markers to geographically link individual Wilson's warblers (*Cardellina pusilla*) captured at a stopover site in the southwestern U.S. during spring migration with their ultimate breeding destination in North America. These studies demonstrate that we can begin to disentangle the complexity of migration when we examine factors known to be important to the success of migration in the context of other periods of the annual cycle. This is a critical step towards understanding population dynamics of migratory species, given that migration is the phase of the annual cycle most often thought to be limiting migratory birds.

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A Dissertation

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CHAPTER I

CLIMATIC VARIABILITY AT OVER-WINTER SITES IN CENTRAL AMERICA
AND SOUTH AMERICA DIFFERENTIALLY AFFECTS INTERCONTINENTAL
MIGRANTS DURING SPRING MIGRATION

Abstract

Predicting how migratory animals will respond to changing climatic conditions requires knowledge of how climatic events affect each phase of the annual cycle and how those effects carry-over to subsequent phases. Research on carry-over effects has largely been limited to the stationary periods, and has rarely considered the role that the migratory period contributes to the overall annual cycle. Yet, the negative impacts of climatic events during the stationary periods may not be manifested until migration, amplifying the risk of mortality during migration, the phase of the annual cycle most often thought to be limiting many migratory species. I utilized a 17-year migration dataset to examine how El Niño-Southern Oscillation climatic events in geographically different regions of the world carry-over to impact the migratory success of several intercontinental migratory bird species. I found that migratory birds that over-wintered in South America experienced significantly drier conditions during El Niño years, as reflected by reduced Normalized Difference Vegetation Index (NDVI) values, and arrived at stopover sites in reduced energetic condition during spring migration. Moreover, during El Niño years migrants were more likely to utilize small forest patches immediately after crossing the Gulf of Mexico where food resources are lower than larger more contiguous forests further inland. In contrast, NDVI values did not differ between El Niño and La Niña years in Caribbean-Central America, and I found no difference in

energetic condition or use of coastal habitats for migrants en route from Caribbean-Central America. Birds over-wintering in both regions had consistent median arrival dates along the northern Gulf coast, suggesting that there is a strong drive for birds to maintain their time program regardless of their overall condition. I directly associated climatic variability experienced at over-winter areas with factors important to the success of migration, providing strong evidence that not only is the biology of migratory landbirds during migration influenced by the previous phase of their life-cycle, but where migratory birds over-winter determines how vulnerable they are to global climatic cycles. My research highlights the importance of considering the entire life-cycle when examining how climatic events influence population dynamics of migratory species.

Introduction

Global climatic patterns and their influence on local weather parameters such as precipitation and temperature can greatly impact biological systems, including the composition and distribution of habitats, as well as determine the abundance of resources available to higher trophic levels (Holmgren et al. 2001, Stenseth et al. 2003). Long-distance migratory animals may be particularly vulnerable to fluctuations in global climate patterns as their seasonal movements between multiple geographic regions increases the likelihood that they will encounter unfavorable or extreme weather patterns at some point during their annual cycle. Further, climatic events that affect an individual during one phase of the annual cycle often carry-over to influence an individual's success in following seasons, resulting in climatic conditions in one part of the world affecting population dynamics in multiple dispersed regions (Sillett et al. 2000, Wilson et al. 2011). The ability to predict how migratory animals will respond to changing climatic

conditions requires integrating how climatic conditions at different phases of the annual cycle interact to influence overall population dynamics (Webster et al. 2002, Moore et al. 2005b, Radchuk et al. 2012). However, establishing how events at any one stage of the annual cycle influence an individual's performance in subsequent seasons is one of the greatest research challenges of our time (Wilcove and Wikelski 2008).

El Niño Southern Oscillation (ENSO) is a major climatic phenomenon influencing weather patterns around the world, and in terrestrial ecosystems exerts both direct and indirect impacts on plant productivity and community dynamics through its effects on precipitation. Periodic oscillations between El Niño (warm state) and La Niña (cold state) episodes results in dramatic increases in rainfall in some regions, while creating severe droughts in other areas (Holmgren et al. 2001). Research examining the impacts of ENSO events on migratory landbird populations has primarily been limited to the stationary periods (i.e. breeding and over-wintering; (Sillett et al. 2000, Nott et al. 2002, Mazerolle et al. 2005, LaManna et al. 2012), while largely overlooking the role that the migratory period contributes to the overall annual cycle (but see Wolfe and Ralph 2009). Yet, for migratory species the negative impacts of climatic events during the stationary periods may not be manifested until migration, amplifying the risk of mortality during migration, the phase of the annual cycle most often thought to be limiting many migratory species (Sillett and Holmes 2002, Newton 2004, Moore et al. 2005a). Climatic models predict the frequency and intensity of ENSO events will increase over the coming decades (Timmermann et al. 1999, Allan and Soden 2008), which could dramatically alter habitat conditions at breeding and over-winter sites from year to year with cascading impacts on the migratory time period, underscoring the critical need for knowledge about

the linkages between climatic events during the stationary phases and factors that influence the success of migration.

Migratory birds are faced with numerous challenges during migration, including increased energy demands, acquisition of food in unfamiliar habitats, predator avoidance, intra- and inter-specific competition for limited resources, abiotic perturbations, and navigational challenges (Moore et al. 1995). These challenges are further intensified by the time constraints associated with migration and the negative fitness consequences that can occur with delays (Møller 1994). The residual effects of events in the previous season can influence how well a migrant responds to the challenges encountered during migration, measured in terms of time and condition (Alerstam and Lindstrom 1990). For example, drier and hotter climatic conditions during the over-winter period may reduce the availability of food resources for migratory birds on their nonbreeding grounds during a time period critical for the preparation of spring migration (Strong and Sherry 2001, Studds and Marra 2007). A migrant departing the nonbreeding grounds with insufficient energy stores does not have a buffer if it encounters difficult conditions such as adverse weather or scarcity of food during stopover, and thus the migrant is less likely to maintain its time schedule (Smith and Moore 2005, Tottrup et al. 2012) and is more vulnerable to mortality. Therefore, carry-over effects of climatic conditions experienced by a migrant during other periods of the annual cycle have the potential to impact the time frame in which a migrant responds to problems encountered during migration, with cascading impacts on survival during migration and future reproductive success.

I utilized a unique long-term migration dataset collected on the northern Gulf of Mexico to understand how ENSO climatic events that differentially impact two over-

winter regions carry-over to affect migratory birds during spring migration. The northern Gulf coast is a major flyway for eastern migratory birds and millions of birds stopover in coastal habitats each spring to rest and refuel after non-stop flights across the Gulf of Mexico (Moore et al. 1995). This study provides a broad-scale examination of how ENSO events in geographically different regions of the world carry-over to impact the migratory success of numerous intercontinental migratory bird species, filling an important gap in our understanding of how global climatic cycles influence population dynamics throughout the annual cycle.

I predicted that El Niño conditions will differentially impact migratory birds overwintering in South America and Caribbean-Central America because of different precipitation and temperature patterns in these regions. During the winter months in northern South America El Niño conditions consistently cause hotter and drier conditions, while La Niña conditions result in cooler and wetter conditions (Ropelewski and Halpert 1987). In contrast, El Niño conditions overall for the Caribbean-Central America region results in only a weak tendency towards drier conditions, especially compared to neighboring regions such as South America (Ropelewski and Halpert 1987). However, localized areas within the Caribbean-Central America region are influenced by El Niño events, particularly regions within the Caribbean and the Pacific coast of Central America (Enfield and Mayer 1997). To directly link ENSO climatic patterns with habitat conditions experienced by migratory birds at over-wintering sites in South America and Caribbean-Central America I measured changes in vegetation vigor via the Normalized Difference Vegetation Index (NDVI) between El Niño, La Niña, and non-ENSO events. Specifically, I predicted limited influence of ENSO events on migratory condition for

birds over-wintering in the Caribbean and Central America given the overall weak impact of ENSO events in these regions. In contrast, I predicted that the drier, warmer El Niño conditions experienced by migratory birds over-wintering in South America will result in: a) Migratory birds having lower energy stores during spring migration. b) Lower energy stores will result in more birds utilizing stopover habitat on the northern Gulf coast immediately after their non-stop flight across the Gulf of Mexico where food resources are lower than in hardwood forests further inland (Buler et al. 2007, Buler and Moore 2011). c) Bird will be less likely to maintain their time program given reduced energy stores, resulting in the overall timing of spring migration being delayed.

Methods

Study Area and Species Selection

I evaluated the potential carry-over effects of ENSO conditions experienced at over-wintering sites on spring migrants at a long-term migration station located along the northern coast of the Gulf of Mexico in Cameron Parish, Louisiana (29°45'N, 93°37'W). Coastal woodlands represent critical stopover habitat for Nearctic-Neotropical landbird migrants (Moore and Kerlinger 1987, Buler et al. 2007), providing the first landfall for eastern migrants in the spring after non-stop flight across this ecological barrier. Birds were captured with mist-nets (12 x 2.6m, 30mm mesh) on a daily basis, 28 March to 6 May, for approximately 8 hrs (0800 to 1700) during all years included in this study (1993 to 1996, 1998 to 2010). Upon capture, birds were banded with a USGS band, age and sex determined according to Pyle (1997), subcutaneous fat assessed according to Helms and Drury (1960), and weighed to nearest 0.1g with an electronic scale. I chose representative species that over-winter in either Central or South America to test the

hypothesis of differential effects of ENSO events in these regions. Within each region, focal species were selected that did not breed at the stopover site and had 30 or more captures per year for at least 10 years of the study (some species captures fall below 30 captures when the overall data set was restricted for some analyses; see Table 1). Focal species over-wintering in Central America were: Hooded Warbler (HWA; *Setophaga citrina*), Indigo Bunting (INBU; *Passerina cyanea*), Kentucky Warbler (KEWA; *Geothlypis formosa*), Ovenbird (OVEN; *Seiurus aurocapillus*), Wood Thrush (WOTH; *Hylocichla mustelina*), while focal species over-wintering in South America were: Gray-cheeked Thrush (GCTH; *Catharus minimus*), Red-eyed Vireo (REVI; *Vireo olivaceus*), Swainson's Thrush (SWTH; *Catharus ustulatus swainsoni*). The over-winter distribution of Swainson's thrush extends across Mexico, Central America, and South America. However, extensive genetic analysis indicates that *C. u. swainsoni*, the subspecies captured at our stopover site, over-winters in South America (Ruegg and Smith 2002).

Spring Migration Variables

I examined three variables, energetic condition, capture rate, and median arrival date, during the migration time period to assess the carry-over effect of ENSO conditions during the over-winter period on migration. First, given the importance of fat reserves for energy during migration (Blem 1990) I calculated the energetic condition of focal species at the stopover site by determining the proportion of body mass attributed to fat by subtracting estimates of fat-free mass from body mass measured at capture (Owen and Moore 2006). Estimates of fat-free mass were calculated

Table 1

Years during the period of the study categorized as El Niño, La Niña, or non-ENSO years based on average Oceanic Niño Index (ONI) values for the winter time period, December to March. ONI values >0.5 represent El Niño conditions, ONI values <-0.5 represent La Niña conditions, and values >-0.5 and <0.5 are categorized as non-ENSO years. The number of each species captured in a given year is indicated, followed in parenthesis by the sample size for only fall out days.

Year	Average winter ONI values	Fall out days	NDVI Central America	Over-winter in Central America					NDVI South America	Over-winter in South America		
				HOWA	INBU	KEWA	OVEN	WOTH		GCTH	REVI	SWTH
<u>El Niño Years</u>												
1995	1.1	6	0.523	38 (15)	75 (21)	30 (10)	31 (17)	40 (18)	0.574	13*	152 (62)	18 (8)
1998	2.2	16	0.518	142 (48)	212 (142)	99 (52)	94 (61)	117 (97)	0.566	103 (65)	182 (128)	120 (69)
2003	1.17	11	0.503	172 (59)	184 (73)	112 (35)	73 (22)	95 (66)	0.563	50 (8)	46 (14)	72 (22)
2005	0.67	8	0.508	74 (7)	115 (53)	46 (9)	63 (32)	145 (99)	0.574	51 (15)	224 (105)	171 (75)
2010	1.67	8		80 (34)	661 (374)	72 (43)	51 (30)	188 (181)		8 (5)	105 (49)	198 (101)
<u>La Niña Years</u>												
1999	-1.3	4	0.517	40*	83 (21)	39 (3)	83 (32)	85 (16)	0.608	33 (9)	76 (26)	67 (13)
2000	-1.5	16	0.533	149 (86)	276 (198)	97 (58)	181 (147)	143 (123)	0.577	59 (37)	112 (89)	91 (73)
2001	-0.6	8	0.5099	108 (38)	197 (79)	102 (56)	64 (34)	81 (41)	0.598	15 (5)	103 (54)	24 (4)
2008	-1.37	6		64 (11)	66 (26)	35 (6)	64 (31)	33 (16)		36 (18)	122 (57)	23 (9)
<u>non-ENSO Years</u>												
1993	0.3	8	0.556	30 (5)	134 (30)	40 (12)	40 (11)	62 (93)	0.598	9 (4)	155 (71)	49 (15)
1994	0.2	8	0.526	7 (4)	95 (50)	28 (15)	31 (14)	13 (9)	0.551	25 (9)	101 (65)	37 (19)
1996	-0.4	8	0.542	39 (7)	96 (30)	26 (10)	37 (10)	57 (14)	0.616	41*	85 (22)	36 (5)
2002	-0.03	6	0.522	59 (11)	83 (23)	42 (10)	74 (40)	65 (18)	0.591	15 (6)	42 (16)	9*
2004	0.37	7	0.507	142 (17)	86 (17)	85 (5)	106 (32)	88 (17)	0.558	47 (12)	89 (39)	77 (40)
2006	-0.47	8	0.522	28 (6)	133 (82)	11 (3)	51 (30)	24 (9)	0.582	44 (17)	46 (20)	48 (17)
2007	0.47	12		159 (72)	71 (21)	47 (17)	48 (22)	64 (25)		22 (11)	92 (45)	48 (18)
2009	-0.4	9		64 (20)	186 (82)	31 (11)	26 (15)	32 (19)		14 (4)	74 (43)	33 (15)

* Data excluded from analysis for energetic condition because less than 3 birds captured under fall out conditions

utilizing the protocol of Ellegren (1992) and Owen and Moore (2006). Larger energetic condition values indicate birds with more fat reserves, and thus greater energetic condition. During spring migration, a bird's energetic state and/or extrinsic factors such as weather constrain how far inland a migrant will travel after crossing the Gulf of Mexico barrier (Gauthreaux 1972, Moore and Kerlinger 1987, Buler and Moore 2011). Birds with remaining fuel reserves are more likely to migrate further inland to larger more contiguous hardwood forests than birds with reduced energetic states (Buler et al. 2007). Therefore, to ensure that the sample was representative of the energetic conditions of all migrants passing through the region, I restricted the analysis to birds captured on "fall out days", which are days when unfavorable weather conditions (e.g.

wind from north, rain) force most birds in both poor and good condition to stop at the stopover site immediately after crossing the Gulf of Mexico (Moore and Kerlinger 1987). Fall out days typically start in the late morning (range 1000 to 1400) when migrants begin to arrive across the Gulf of Mexico (Gauthreaux 1971, 1972), hence I defined a fall out day as having greater than 50 new captures after 1100 hours. Only birds newly captured after 1100 were included in the analysis to avoid including birds that remained at the site but were not captured on previous banding days.

Second, I calculated a rate of capture to assess if potential variation in energetic condition between El Niño and La Niña years resulted in differences in the number of birds utilizing the stopover site. I utilized the entire migration season for this analysis given the strong influence of weather on capture rates during fall out days. I defined the rate of capture as the number of individuals per focal species captured in a season for every 100 net hours.

Last, I calculated the median date of arrival for each season per focal species to determine if the timing of spring migration varies in relation to El Niño conditions experienced at over-winter sites. Median date of arrival was utilized because it is unaffected by outliers and therefore would not be biased by extremely early or late migrants within a given year. The entire season was utilized for this calculation because fall out days are not necessarily evenly dispersed across the migration season.

ENSO Classification

I identified years between 1993 and 2010 representing episodes of El Niño or La Niña conditions during the winter time period utilizing the National Center for Environmental Predictions Oceanic Niño Index (ONI;

http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml), which is a measure of the departure from long-term averages of seas surface temperatures (SST) in the east-central Pacific Ocean, specifically the Niño 3.4 region (5°N-5°S, 120°-170°W). The index classifies El Niño and La Niña events based on a threshold of 3 consecutive months above the 0.5°C SST anomaly (El Niño) or below the -0.5°C SST anomaly (La Niña). We examined ONI values between December and March, and included only years meeting the threshold for El Niño or La Niña conditions.

ENSO Impact on Vegetation Vigor

To directly link fluctuations in ENSO weather patterns with changes in habitat conditions experienced by focal species at their over-winter sites in Central and South America, I examined differences in NDVI values between El Niño, La Niña, and non-ENSO years for species-specific over-winter ranges. NDVI values, a measure of vegetation on the land surface assessed from remotely sensed imagery, provide a simple measure of the amount of vegetation vigor and density, with higher NDVI values reflecting more rainfall and increased primary productivity (Tucker et al. 1991). I obtained NDVI values from the Global Inventory Modeling Mapping Studies (GIMMS) dataset that spans from 1981 to 2006 (Tucker et al. 2004) and is derived from imagery obtained from the Advanced Very High Resolution Radiometer (AVHRR) sensor onboard the National Oceanic and Atmospheric Agency (NOAA) satellites (15 day composites at 1° resolution). The GIMMS data set is corrected for calibration, view geometry, cloud cover, and other effects not related to vegetation change (Pinzon et al. 2005, Tucker et al. 2005). I defined over-winter ranges of our focal species in each region using wintering ranges from (Ridgley et al. 2003). Utilizing ArcGIS version 10.0

we obtained NDVI values for each species' over-winter range between the time period of 15 February to 31 March, a critical time period during the nonbreeding season during which migrants prepare for migration (Studds and Marra 2007, 2011, Wilson et al. 2011, McKellar et al. 2013). I then calculated a mean NDVI value for each species for each spring (e.g. February & March) time period.

Statistical Analysis

I performed all statistical analyses in R version 3.0.1 (R Development Core Team 2013). Using the R package 'lme4' (Bates and Maechler 2011) I constructed Linear Mixed Models (LMM) using a Gaussian distribution and identity link function. Separately for each over-winter region (South and Central America), I used LMM to test for differences in species-specific NDVI values as a function of ENSO condition (El Niño, La Niña, or non-ENSO) with species as a random factor. For statistical analyses of bird-related response variables, mean values for each species per year were utilized to control for differences in the number of each species captured and variation in the number of fall out days between years. Thus patterns were not driven solely by large samples in a particular year or species. To determine whether ENSO conditions influenced energetic condition, capture rate, and median arrival date at a migratory stopover site, I utilized LMM. I modeled each response variable (energetic condition, capture rate, median arrival date) as a function of ENSO condition (El Niño, La Niña, non-ENSO) separately for both Central and South America. Models included species (5 species in Central America, 3 species in South America) as a random effect to control for distinct differences among species in fat loads, capture rates, and timing of arrival at the stopover site. I evaluated the significance of each model using an ANOVA function in

the R package lmerTest. The approximation for the denominator degrees of freedom of the F statistic is Satterthwaite's, a calculation based on SAS proc mixed theory (Bates and Maechler 2011). For significant models, we also calculated parameter estimates and standard error and accompanying p-values for fixed factors of the LMM using the lmerTest package in R.

Results

Nine years met the threshold for El Niño ($n=5$) and La Niña conditions ($n=4$) during the time period of the study (1993 to 1996, 1998 to 2010; Table 1). In South America, El Niño conditions resulted in significantly reduced NDVI values, a measure of primary productivity, across each focal species' over-winter range compared to La Niña years (Figure 1a, Table 2; $n=39$, $F=3.91$, $p=0.03$). During non-ENSO years, average NDVI values across species' over-winter ranges in South America did not differ from La Niña years (Figure 1a, Table 2). As predicted, vegetation vigor did not differ between El Niño and La Niña years in Central America; however, NDVI values were highest during non-ENSO years (Figure 1b; $n=65$, $F=8.09$, $p=0.0001$).

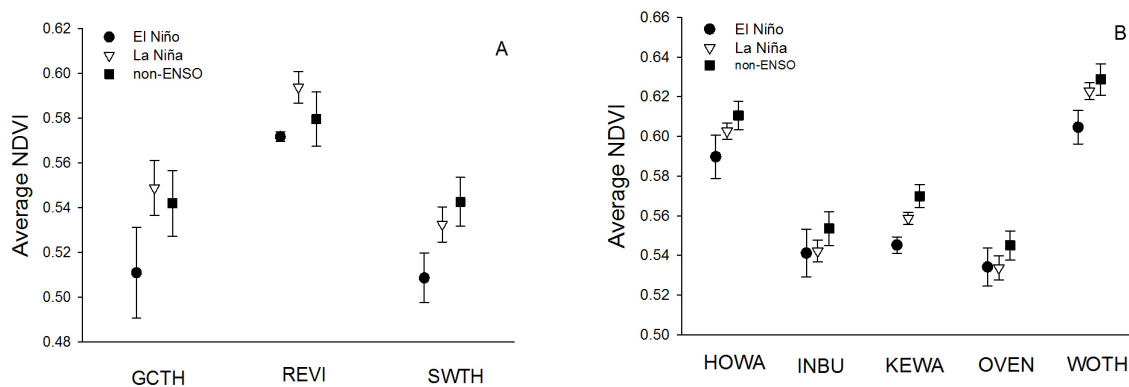


Figure 1. Comparison between El Niño, La Niña, and non-ENSO years of average Normalized Difference Vegetation Index (NDVI) values for each species' over-winter

range between the time-period of 15 February to 31 March. Average NDVI values between El Niño and La Niña years was significantly different for species over-wintering in (A) South America (El Niño: $x = 0.53 \pm 0.01$, La Niña: $x = 0.56 \pm 0.01$), while average NDVI values during non-ENSO years ($x = 0.55 \pm 0.008$) were consistent with La Niña years. Species over-wintering in (B) Caribbean-Central America did not differ in average NDVI values between El Niño and La Niña years (El Niño: $x = 0.56 \pm 0.008$, La Niña: $x = 0.57 \pm 0.009$), but both El Niño and La Niña years differed from non-ENSO years ($x = 0.58 \pm 0.007$).

Table 2

Summary of parameter estimates, standard error, t-value, and p-value based on Linear Mixed Models testing for differences in species-specific NDVI values, a bird's condition at arrival at the stopover site, capture rate (number of birds/100 net hours), and median arrival date at the stopover site as a function of El Niño, La Niña, and non-ENSO conditions in South America. The value in parentheses following ENSO category indicates the category modeled.

Model	Parameter Estimate	Standard Error	t	p
NDVI				
ENSO(El Niño)	-0.03	0.01	-2.39	0.02
ENSO(non-ENSO)	-0.003	0.01	-0.34	0.74
Condition				
ENSO(El Niño)	-0.98	0.39	-2.5	0.02
ENSO(non-ENSO)	-0.1	0.36	-0.28	0.78
Capture Rate				
ENSO(El Niño)	0.63	0.29	2.15	0.04
ENSO(non-ENSO)	0.16	0.27	0.59	0.56
Median Arrival				
ENSO(El Niño)	-2.38	2.16	-1.1	0.28
ENSO(non-ENSO)	-4.85	1.11	-2.46	0.02

Consistent with the prediction I found that in El Niño years focal species over-wintering in South America had lower energetic condition values (Figure 2a, Table 2; $n=48$, $F=4.22$, $p=0.02$) and higher capture rates at the stopover site (Figure 3a, Table 2; $n=51$, $F=2.71$, $p=0.06$) compared to La Niña and non-ENSO years. Energetic conditions and rates of capture during non-ENSO years were consistent with La Nina years (Figure

2a & 3a, Table 2). During years with El Niño conditions on the nonbreeding grounds, energetic condition of birds en route from South America was almost half the value of birds captured during La Niña years (El Niño: $x = 1.52 \pm 0.29$, La Niña: $x = 2.49 \pm 0.32$), with twice as many birds utilizing the coastal stopover site (El Niño: $x = 1.54 \pm 0.29$, La Niña: $x = 0.81 \pm 0.14$). Contrary to the prediction, reduced energetic condition during El Niño years did not result in a later median arrival date for focal species at the stopover site compared to La Nina and non-ENSO years; however, birds arrived significantly earlier during non-ENSO years (Figure 4a, Table 2; $n=51$, $F=3.15$, $p=0.05$).

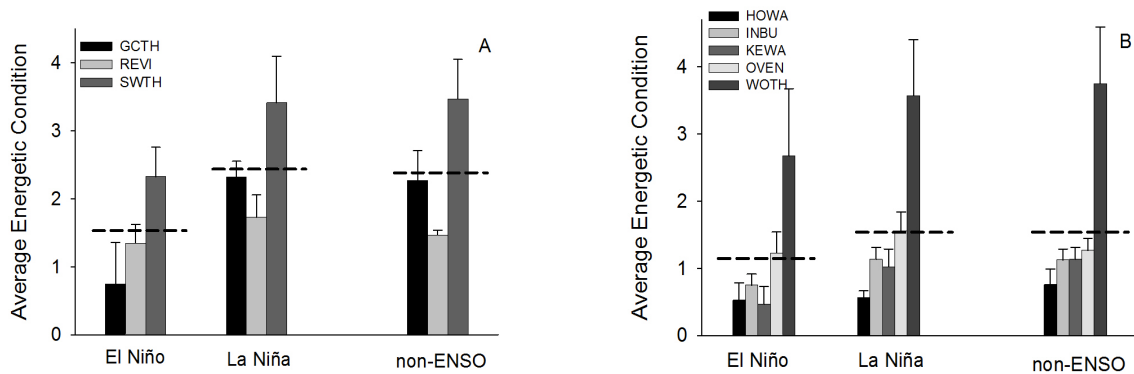


Figure 2. Comparison of the average energetic condition of stopover migrant species that over-wintered in (A) South and (B) Caribbean-Central America during El Niño, La Niña, and non-ENSO years. Error bars represent \pm SE and dashed lines represent average values for each region (South America: El Niño: $x = 1.52 \pm 0.29$, La Niña: $x = 2.49 \pm 0.32$, non-ENSO: $x = 2.36 \pm 0.29$; Central America: El Niño: $x = 1.12 \pm 0.26$, La Niña: $x = 1.65 \pm 0.32$, non-ENSO: $x = 1.63 \pm 0.24$). There were significant differences between El Niño and La Niña years in the average energetic condition for species over-wintering in South America, and average energetic condition during non-ENSO years were consistent with La Niña years. Energetic condition did not differ between El Niño, La Niña, and non-ENSO years for species over-wintering in Caribbean-Central America region.

In contrast to birds over-wintering in South America, we found that energetic condition (Figure 2b; $n=83$, $F=1.88$, $p=0.16$) and median arrival date (Figure 4b; $n=85$, $F=1.92$, $p=0.15$) did not differ between El Niño, La Niña, and non-ENSO years for

species over-wintering in Caribbean-Central America. The overall model for capture rate indicated significant differences in capture rates between ENSO conditions (Figure 3b; $n=85$, $F=3.15$, $p=0.05$). However, one extreme outlier (INBU 2010 capture rate: 8.17 birds/100 net hours) was driving the significant pattern. When I removed the outlier the pattern was no longer significant ($n=84$, $F=2.26$, $p=0.11$).

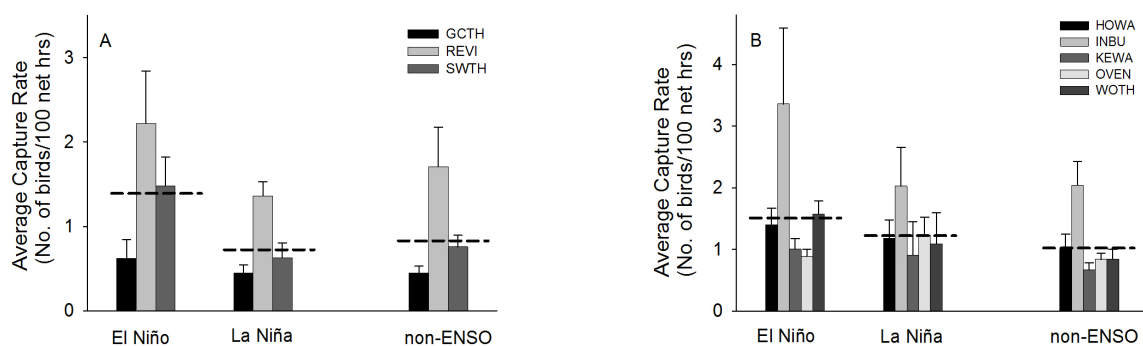


Figure 3. Comparison of average capture rate of stopover migrant species that over-wintered in (A) South and (B) Caribbean-Central America during El Niño, La Niña, and non-ENSO years. Error bars represent \pm SE and dashed lines represent average values for each region. There were significant differences between El Niño and La Niña years in the average capture rate for species over-wintering in South America, and average capture rate during non-ENSO years were consistent with La Nina years. Capture rates did not differ between El Niño and La Niña years for species over-wintering in Caribbean-Central America region.

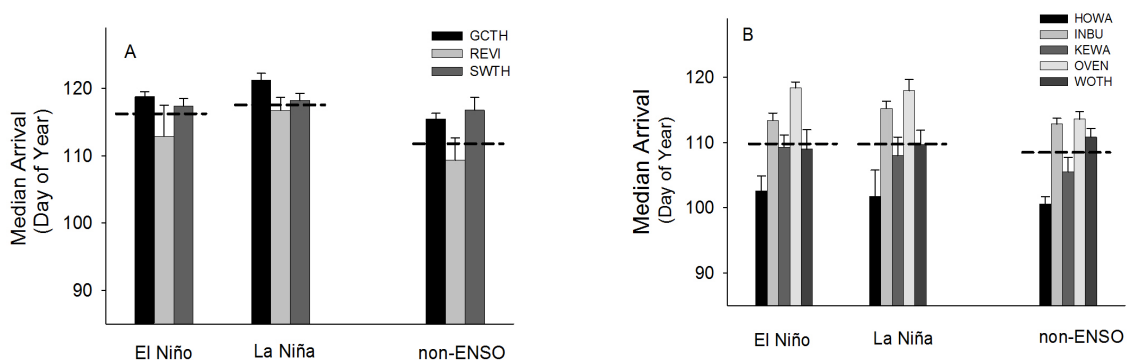


Figure 4. Comparison of median arrival date of stopover migrant species that over-wintered in (A) South and (B) Caribbean-Central America during El Niño, La Niña, and

non-ENSO years. Error bars represent \pm SE and dashed lines represent average values for each region (South America: El Niño=116 \pm 1.64, La Niña=118 \pm 0.92, non-ENSO: $x=113.90\pm1.41$; Central America: El Niño=110.54 \pm 1.34, La Niña=110.55 \pm 1.66, non-ENSO: $x=108.7\pm0.99$). Range of median arrival dates, April 1 = Day 91 to May 25 = Day 125. There was no significant difference between El Niño and La Niña years in median arrival date for either region. However, for species over-wintering in South America, median arrival date differed significantly between La Niña and non-ENSO years.

Discussion

ENSO conditions, which cause regional variation in precipitation and temperature, differentially affected migratory birds at over-winter sites in Central and South America with cascading impacts during spring migration. Migratory birds that over-wintered in South America experienced significantly hotter and drier conditions during El Niño years (Ropelewski and Halpert 1987, Holmgren et al. 2001), which was verified by the NDVI analysis indicating a reduction in primary productivity during El Niño years compared to La Niña and non-ENSO years. In contrast, NDVI values did not differ between El Niño and La Niña years in Caribbean-Central America, which is consistent with only a weak tendency towards drier conditions overall in Caribbean-Central America during El Niño years (Ropelewski and Halpert 1987, Holmgren et al. 2001). Reduced primary productivity during El Niño conditions, as reflected by low NDVI values, presumably cascades to depress arthropod abundances (Pearson and Derr 1986, Poulin et al. 1992, Ahumada 2001), which reduces availability of food resources to migratory birds during the critical pre-migratory time period when migrants are building energy reserves for migration. Therefore, I expected migratory birds over-wintering in areas where primary productivity is influenced by ENSO to depart with lower energetic condition during El Niño years. The results were consistent with this expectation. I found reduced energetic condition during migration in El Niño years for those migrants

over-wintering in South America, and no difference in energetic condition during migration between El Niño, La Niña, and non-ENSO years for migrants over-wintering in Central America.

Despite the complex interaction of many factors that can influence energetic condition, the clear relationship we found between ENSO conditions during the over-winter period in South America and a migrant's condition during spring migration provides strong evidence that climatic conditions experienced during the stationary, non-breeding phase of the annual cycle have consequences that carry-over to affect a bird's condition during migration (see also Wolfe and Ralph 2009). A bird's energetic state during migration directly impacts how well a migrant negotiates the challenges of migration (Moore et al. 2005b), including aspects of foraging and predator avoidance. Whereas many challenges encountered during migration such as food acquisition and competition for limited resources are similar to challenges faced throughout the annual cycle, the energy demands as well as time and information constraints imposed on birds during the migratory phase amplify these challenges (Moore and Kerlinger 1990, Moore et al. 2005b). Therefore, birds departing over-wintering sites with insufficient energy stores to meet the considerable physiological challenges of migration may experience a higher risk of mortality, especially when crossing an ecological barrier such as the Gulf of Mexico.

Consistent with our prediction, I did not find a clear pattern between ENSO conditions during the over-winter time period in Caribbean-Central America and a bird's energetic condition during spring migration. However, localized populations within these over-winter regions may have been impacted by El Niño events with carry-over effects

during the migratory time period. For example, Sillett et al. (2002) found that Black-throated blue warblers (*Setophaga caerulescens*) over-wintering in Jamaica had reduced annual survival during El Niño years (Sillett et al. 2002), and that the majority of mortality for Black-throated blue warblers was concentrated during the migratory time period (Sillett and Holmes 2002). While the overall impact of ENSO events in Central American region is weak compared to other regions (Ropelewski and Halpert 1987), the extent to which regions within the Caribbean and Central America are affected by ENSO conditions varies. In addition, understanding the impacts of ENSO conditions in the Caribbean and Central America is further complicated by the topography of the region, which results in contrasting climatic conditions along the Caribbean (increased rainfall) and Pacific (decreased rainfall) coasts during El Niño events (Enfield and Mayer 1997). The impact of El Niño events on some populations over-wintering in the Caribbean-Central America is potentially diffused at our study site by the diversity of breeding populations from a wide range of over-winter regions utilizing coastal habitats on the northern Gulf of Mexico.

Energetically constrained migrants are often forced to make landfall in the first areas encountered after crossing an ecological barrier, regardless of habitat suitability (e.g. Kuenzi et al. 1991, Barriocanal and Robson 2007). During El Niño years, birds en route from South America were twice as likely to utilize habitat immediately along the Gulf coast after their non-stop flight across the Gulf of Mexico. Within the heavily urbanized Gulf coast region a mosaic of small isolated patches of habitat provide migratory birds with a refuge to rest after crossing the Gulf of Mexico. However, food resources to replenish fat and muscle reserves within these small patches are lower than

in larger more contiguous hardwood forests further inland (Buler et al. 2007). Moreover, as birds concentrate within these small-forest patches, competition for already limited resources increases and fuel deposition rates decline (Moore and Wang 1991, Kelly et al. 2002a). Thus during El Niño years, reduced energetic condition during migration resulting from adverse climatic conditions experienced during the over-winter time period may be compounded by landfall in less suitable habitat, imposing additional costs during the migratory time period with cascading impacts on survival and future reproductive performance.

The median arrival date was remarkably consistent during the 17-year study, despite climatic variability during the over-winter phase of the annual cycle, suggesting that there is a strong drive for birds to maintain their time program during migration (Alerstam and Lindstrom 1990, Hedenstrom and Alerstam 1997, Stanley et al. 2012), likely in response to the enhanced reproductive success of early arriving individuals (Møller 1994, Saino et al. 2004, Norris et al. 2004, Smith and Moore 2005). This consistency is contrary to the prediction that the cascading impact of reduced energy stores for birds over-wintering in South America during El Niño years would result in later arrival at stopover sites along the Gulf coast. Whereas transitions between different phases of the annual cycle may be controlled by an endogenous time program synchronized with seasonal changes in photoperiod (Berthold 1995), there is evidence that spring migration is phenotypically plastic among some intercontinental migratory species, and climatic conditions such as reduced precipitation at nonbreeding sites may delay the timing of spring departure (Saino et al. 2004, 2007, Studds and Marra 2011). If a bird is to maintain its endogenous time program we might expect initiation of migration

to vary little from year to year, even when climatic conditions depress arthropod abundances, and consequently affect the margin of safety in energy reserves for departing migrants. That said, if migrants are not able to compensate for the reduced energy reserves during the migratory period, they are likely to arrive at their breeding grounds without surplus fat stores, and may suffer reproductive consequences (Smith and Moore 2003). Alternatively, El Niño conditions may delay spring departure from over-wintering areas as birds forage longer to gain sufficient energy stores, forcing birds to increase their speed of migration to stay on schedule with their time program. While few studies have been able to quantify the rate of migration for small landbirds, given the challenges of following individuals across large spatial scales (but see Wikelski et al. 2003, Stutchbury et al. 2009), evidence suggests that birds can adjust the rate of migration in response to ecological conditions during migration (Marra et al. 2004, Robson and Barriocanal 2010, Tottrup et al. 2012). However, even with delayed departure from the nonbreeding grounds, birds may still have reduced energy reserves if arthropod abundances are sufficiently depressed.

If we are to understand how climatic events impact migratory populations, we must integrate the cumulative effects of events that occur during different phases of the annual cycle. While studies have found associations between ENSO events on the breeding and nonbreeding grounds with annual survival, productivity, and recruitment (Silleet et al. 2000, Nott et al. 2002, Mazerolle et al. 2005), rarely have studies considered the influence of the migratory period on the overall annual cycle. Yet, the negative impacts of climatic events during the stationary, nonbreeding phase of the annual cycle may not be manifested until migrants are faced with the physiological demands of

migration. Climatic models predict that the frequency and intensity of ENSO events will increase in the coming decades (Timmermann et al. 1999, Allan and Soden 2008). Increased El Niño events, coupled with the loss of stopover habitat, especially within coastal regions (Buler and Moore 2011), may intensify the challenges of migration, increasing the risk of mortality during migration. The differential impact of El Niño conditions on the migratory condition of birds over-wintering in Central and South America, suggests that more frequent El Niño events may disproportionately influence long-distance migrants over-wintering in South America. We may expect that La Niña years, which have the opposite climatic effects (e.g. wet and cool) of El Niño conditions in South America (Ropelewski and Halpert 1987, Holmgren et al. 2001), might provide a positive response to birds to offset the negative effects of El Niño years. This prediction is consistent with studies that found higher reproductive success for breeding birds in North America during La Niña years, presumably due to increased food resources (Silleet et al. 2000, Mazerolle et al. 2005). Yet, I found that the energetic condition of birds over-wintering in South America during La Niña years largely overlapped with non-ENSO or ‘normal years’, as did NDVI values, suggesting that increases in rain above a certain level may not necessarily increase primary productivity and food resources for bird species over-wintering in the tropical forests of South America. Therefore, increased frequency of ENSO events are likely to have mostly negative impacts on the population dynamics of bird species over-wintering in South America.

The phases of a migrant’s annual cycle are necessarily linked. Our understanding of the migratory time period has been limited by the difficulty of determining a migrant’s geographic linkage to over-wintering and breeding areas and the conditions a migrant

experiences prior to arriving at a stopover site. There is increasing evidence that late winter climate influences the time of arrival on breeding grounds and subsequent reproductive success for long-distance migrants (Studds and Marra 2007, Wilson et al. 2011, Rockwell et al. 2012, McKellar et al. 2013), but little is known about how climate during the over-winter period may influence migrants during migration, the phase of the annual cycle most often thought to be limiting migratory bird populations (Silleet and Holmes 2002, Newton 2004, Moore et al. 2005a). I provide the strong evidence that not only are migratory birds during spring migration influenced by events occurring during the previous phase of their annual cycle, but where they over-winter determines how vulnerable they are to global climatic cycles. I directly associate climatic variability experienced at over-winter areas with factors important to the success of migration, namely timing and condition, providing important insight into how global climatic cycles such as ENSO influence population dynamics throughout the annual cycle. Predicting how migratory species will adapt to a changing climate, including increased frequency and intensity of ENSO events (Timmermann et al. 1999, Allan and Soden 2008), requires understanding how climate effects each phase of the annual cycle and how those effects carry-over to subsequent phases.

CHAPTER II

SEASONAL INTERACTIONS: THE INFLUENCE OF OVER-WINTER HABITAT
QUALITY ON THE SUCCESS OF MIGRATION

Abstract

I examined how conditions prior to the onset of migration influenced migration success for multiple breeding populations of black-and-white warblers (*Mniotilta varia*) captured during spring migration by linking information on the migrant's winter habitat quality, measured via stable carbon isotopes, with information on their breeding area destination, measured via stable hydrogen isotopes. The quality of over-winter habitat strongly influenced the timing of migration for black-and-white warblers when I accounted for differential timing of migration between breeding populations. Among birds migrating to the same breeding destination, males and females arriving early to the stopover site originated from higher-quality habitat than later arriving birds, suggesting that the benefits of occupying high-quality habitat during the over-winter time period carry-over to positively influence the timing of migration. However, contrary to my expectation, male warblers arriving early to the stopover site were not in better migratory condition than later arriving conspecifics that originated from poor-quality over-winter habitat. This pattern was the same for birds migrating to breeding area destinations in both the southeastern U.S. and the boreal forest of Canada, which stopover at the study site during different time periods, suggesting that the reduced migratory condition of early birds is not a function of the time of season, but a migrant's migration strategy. Strong selection pressures to arrive early on the breeding grounds to secure high-quality breeding territories may drive males from high-quality winter habitat to minimize time at

the expense of energy, resulting in a smaller margin of safety to buffer the effects of adverse weather or scarcity of food, increasing the risk of mortality during migration. In contrast, the migratory condition of females was the same regardless of the timing of migration or breeding area destination, suggesting that females adopt a migration strategy of minimization of energy consumption in order to arrive on the breeding grounds with excess fat reserves. Understanding how events prior to migration influence the success of migration is critical to understanding population dynamics of migratory species.

Introduction

The biology of migratory species, their life histories, adaptations, and population dynamics, is shaped by all phases of the annual cycle and the geographic connectivity among phases (Webster et al. 2002, Greenberg and Marra 2005, Moore et al. 2005b). Moreover, events in different phases of the annual cycle can interact so that residual effects from one season carry-over to influence an individual's success in subsequent seasons (Webster et al. 2002, Harrison et al. 2010). Therefore, to understand the biology of migratory species I must adopt a cross-seasonal approach to understand how phases of the annual cycle interact with one another (Greenberg and Marra 2005). Of course, a serious challenge to understanding seasonal interactions of migratory species is the difficulty in following individuals throughout their annual cycle and tracing the impacts of carry-over effects between phases of the annual cycle that occur across dispersed geographic areas.

Carry-over effects are likely more widespread than currently indicated (Norris and Marra 2007, Harrison et al. 2010), but only recently with advances in the use of intrinsic markers, such as stable isotopes, have seasonal interactions been document for small

landbird migrants. Studies using stable isotopes have increased our understanding of connections between the breeding and over-winter periods (Boulet et al. 2006, Hobson et al. 2010), patterns of migration (Kelly et al. 2002b, 2005, Mazerolle et al. 2005, Paxton et al. 2007), and seasonal interactions between phases (Saino et al. 2004, Reudink et al. 2009, Wilson et al. 2011, Studds and Marra 2011, McKellar et al. 2013). In addition, studies using stable carbon isotopes ($\delta^{13}\text{C}$) have revealed that the quality of over-winter habitat influences a bird's stress hormones (Marra and Holberton 1998), physical condition (Marra et al. 1998, Strong and Sherry 2001, Studds and Marra 2005, Thomas 2012), and spring departure dates (Marra et al. 1998, Studds and Marra 2011), and that the consequences associated with over-winter habitat quality carry-over to affect an individual's timing of arrival at breeding destinations with cascading impacts on reproductive success (Marra et al. 1998, Norris et al. 2004, Reudink et al. 2009). While studies linking carry-over effects from over-winter habitat to reproductive success on the breeding grounds have dramatically increased our understanding of seasonal interactions, they largely ignore the intervening migratory time period, and the influence of events en route in shaping the timing of arrival at breeding destinations and the cascading impacts on reproductive success. Moreover, these studies only account for birds that survived migration. Yet, timing of arrival at breeding destinations is most likely influenced not only by conditions on the non-breeding grounds, but also the cumulative effect of experiences encountered during spring migration and the interaction between the over-winter and migratory phases.

Research examining seasonal interactions has primarily been limited to linking the stationary periods (Marra et al. 1998, Saino et al. 2004, Reudink et al. 2009), while

largely overlooking the role that migration contributes to the overall annual cycle (but see Bearhop et al. 2004, Moore et al. 2005b, González-Prieto and Hobson 2013). The lack of studies demonstrating seasonal interactions between stationary and migratory period is largely a function of the difficulty in determining a migrating bird's geographic linkage to over-wintering and breeding areas and the conditions a migrant experiences prior to arrival at a stopover site. Yet, the migratory time period is one of the most energetically expensive time periods of the annual cycle (Blem 1990) and the residual effects of events in the previous season has the potential to influence how well a migrant negotiates the physiological challenges encountered during migration, the phase of the annual cycle most often thought to be limiting many migratory species (Silleet and Holmes 2002, Newton 2004, Moore et al. 2005a).

In this study, I examined how conditions prior to the onset of migration influence migration success for multiple breeding populations of black-and-white warblers (*Mniotilta varia*) captured during spring migration. Specifically, I linked information on the migrant's winter habitat quality, measured via $\delta^{13}\text{C}$, with information on their breeding area destination, measured via stable hydrogen isotopes (δD), to understand how factors associated with the stationary periods influence the timing and condition of birds during migration. Seasonal interactions are generally manifested when there is variation in resource availability, resulting in individuals transitioning between seasons at differing levels of physical condition (Harrison et al. 2010). The annual dry season (January to April) in most of the Caribbean Islands and Latin America coincides with the end of the over-winter period and the onset of migration, resulting in a reduction in insect biomass at a time when migratory birds need to accumulate fat in preparation for

migration (Strong and Sherry 2001, Brown and Sherry 2006, Studds and Marra 2007). Variation in the amount of food resources between mesic and xeric habitat during the dry season, coupled with increased energy demands, results in habitat-specific differences in the quality of over-winter sites for black-and-white warblers (Thomas 2012) and other Neotropical migrants such as American redstart (*Setophaga ruticilla*; Marra et al. 1998, Studds and Marra 2005), northern waterthrush (*Parkesia noveboracensis*; Smith et al. 2010) and Ovenbird (*Seiurus aurocapillus*; Strong and Sherry 2001, Brown and Sherry 2006). Therefore, the quality of a black-and-white warbler's over-wintering habitat sets the stage for spring migration. For example, a migrant in poor-quality over-winter habitat may require more time for fat deposition than conspecifics in higher-quality habitat, delaying departure from the wintering grounds, and compromising the optimal migratory schedule. Delays in departure from the wintering grounds can have cascading consequences during migration as resource levels at stopover sites may be depressed by earlier migrants (Moore and Wang 1991), further delaying the pace of migration because of longer refueling periods. Moreover, a migrant with insufficient energy stores is not buffered against difficult conditions such as adverse weather or scarcity of food encountered during stopover, placing further pressure on the migrant's ability to maintain its time schedule (Smith and Moore 2005, Tottrup et al. 2012) not to mention exposure to heightened risk of mortality.

I expect consequences associated with habitat quality on black-and-white warbler's over-wintering grounds (Thomas 2012) to carry-over to affect an individual's success during migration, measured in terms of time and condition (Alerstam and Lindstrom 1990). Understanding differences in the quality of over-winter habitats of

black-and-white warblers and the consequences of habitat occupancy on body condition (Thomas 2012) allows me to make specific predictions about carry-over effects from the over-winter time period during spring migration. Additionally, stable hydrogen isotope analysis identifies the breeding destination of black-and-white warblers as they migrate through the stopover site, which controls for population-specific differences in timing of migration. Specifically, I predicted that individuals originating from high-quality over-winter habitat will (1) arrive earlier to the stopover site, and (2) be in better migratory condition compared to conspecifics migrating to the same breeding area destination.

Methods

Study site and species

Black-and-white warblers were captured during spring migration 2008 to 2011 with mist-nets (12 x 2.6m, 30mm mesh) on a daily basis, 21 March to 13 May, for approximately 8 hrs (0800 to 1700) at a long-term migration banding station located along the northern coast of the Gulf of Mexico in Cameron Parish, Louisiana (29°45'N, 93°37'W). Upon capture, birds were banded with a USGS band and a unique color band combination, age and sex determined according to Pyle (1997), and weighed to nearest 0.1g with an electronic scale. Additionally, two tail feathers (R5) were pulled for δD analysis and a blood sample via brachial vein puncture was taken for $\delta^{13}C$ analysis (red blood cells). Blood samples were stored on ice, and then frozen within 6 hours of collection. In 2010 and 2011, 2 to 3 mm from the central claw of each foot was also clipped for $\delta^{13}C$ analysis.

Black-and-white warblers winter range extends across Caribbean-Central America region and northern South America (Kricher 1995). While the specific winter

origin of warblers captured at the stopover site is not known, studies using δD indicate a strong connectivity between populations on the wintering and breeding grounds (Marra and Studds 2013). Black-and-white warblers exhibit a chain migration where warblers in the western winter range (e.g., Mexico, Belize, Nicaragua) migrate to breeding locations at northern latitudes in central Canada, and warblers in the eastern winter range (e.g., Dominican Republic, Jamaica, Puerto Rico, and St. Martin) migrate to breeding locations at more southern latitudes in the U.S (Marra and Studds 2013). Thus, warblers captured at the stopover site migrating to breeding area destinations in the southeastern U.S. and boreal forest in Canada most likely originated from the eastern Caribbean Islands and Central America, respectively (Marra and Studds 2013). Black-and-white warblers do not over-winter or breed at the study site; so all warblers captured were transients.

Over-winter Habitat Quality Determination

Stable-carbon isotope signatures of plants in the tropics vary by habitat type as a result of differences in photosynthetic pathways (C3 vs C4) and water-use efficiency of the dominant plant species (Marshall et al. 2007). Differences in water availability and plant water stress in habitats occupied by black-and-white warblers results in habitat-specific $\delta^{13}C$ signatures, similar to studies of American redstarts (Marra et al. 1998). Black-and-white warblers incorporate $\delta^{13}C$ values of their environment into their tissues through consumption of phytophagous insects and therefore carry an isotopic signature identifying their over-winter habitat. Therefore, black-and-white warblers originating from high quality over-winter habitats which tend to be moister (e.g., wet and mangrove forest) will have more depleted $\delta^{13}C$ values, whereas more enriched $\delta^{13}C$ values will

indicate over-winter occupancy of low quality habitat (e.g., scrub forest; Marra et al. 1998).

Diet switching experiments in multiple bird species have shown that red blood cells have a turnover rate of six to eight weeks (Hobson and Clark 1993, Pearson et al. 2003, Hobson and Bairlein 2003), while claws are metabolically inert after formation and the tip of the claw represents 2 to 5 months prior to sampling (Bearhop et al. 2003). Thus $\delta^{13}\text{C}$ values from red blood cells and claw samples of black-and-white warblers captured during spring migration should represent a habitat-specific $\delta^{13}\text{C}$ signature of their over-winter habitat. To verify that $\delta^{13}\text{C}$ values from red blood cells reflect over-winter habitat I compared $\delta^{13}\text{C}$ values from claw and red blood cells collected from the same individuals ($n=55$, $r=0.74$, $p=0.001$). Given the strong relationship between red blood cells and claw samples, and that I had red blood cell samples from individuals across all four years of the study, I utilized only $\delta^{13}\text{C}$ values of red blood cells for all subsequent analyses.

Breeding Area Destination

Direct assignment to a geographic location utilizing only δD values from feathers is not reliable because of inter-individual differences in physiology, analytical error, and error associated with the isoscape to which birds are assigned (Wunder and Norris 2008). Therefore, I created a probabilistic surface of breeding area destinations for a range of δD values (southeast: -10‰ to -50‰ , boreal forest: -70‰ to -130‰) utilizing information from both the sample and error associated with δD values of feathers and precipitation (Hobson et al. 2009a, 2009b) to determine the probability that the breeding area of an individual black-and-white warbler captured at the migration station was from a

particular breeding area destination (southeast or boreal forest). I adjusted stable feather isotope values (δD_f) of warblers captured at the stopover site to reflect stable hydrogen isotope values of precipitation (δD_p) utilizing the equation: $\delta D_f = 0.95 (\delta D_p) - 17.57$ (non-ground foraging Nearctic-Neotropical migrants; Hobson et al. 2012). To calculate an estimate of error I determined within-site standard deviations (s.d.) of δD values from fifty-five Wilson's warbler (*Cardellina pusilla*) and MacGillivray's warbler (*Geothlypis tolmiei*) feather samples collected from 10 locations across their breeding ranges (data from Paxton et al. 2007). To visualize the likely point of origin for the ranges of δD values specified above, similar to Hobson et al. (2009a), I utilize Program R to generate a random sample of 1000 possible isoscapes values for each isotope value considered using a normal distribution with the isotope values as the mean and the average error (5.94 s.d.) generated from Paxton et al. (2007) above. A normal distribution was fit to the entire simulated dataset, and a probability of belonging to the chosen range of δD values was calculated for all deuterium values in the isoscape. Using the calculated probability densities I then reclassified the (Bowen et al. 2005) isoscape using Spatial Analysis in ArcGIS v9.1 (ESRI, Redlands, CA) for each breeding area destination. BAWW with δD values between -10‰ to -50‰ and -70‰ to -130‰ had the highest probability of breeding in the southeast and boreal forest (Figure 5), respectively, and thus only black-and-white warblers with δD values within these ranges were used in subsequent analyses.

Stable Isotope Analysis

All stable isotope analyses were conducted at the University of Oklahoma. Stable isotope ratios are expressed in standard δ notation, where $\delta^{13}\text{C}$ or $\delta D = [(\text{isotope ratio}_{\text{sample}}/\text{isotope ratio}_{\text{standard}}) - 1] \times 1000$. Consequently, $\delta^{13}\text{C}$ and δD are expressed in

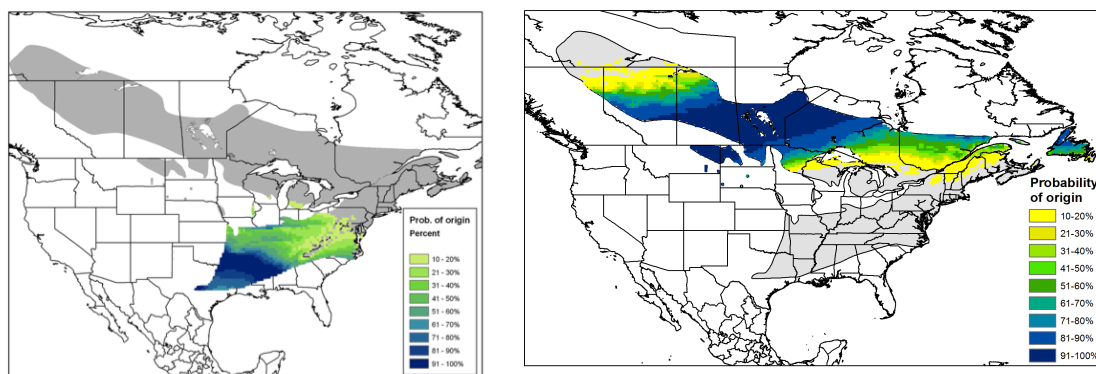


Figure 5. Black-and-white warbler breeding range showing the probability of breeding origin for the (A) southeastern U.S. (δD values between -10‰ and -50‰) and (B) boreal forest (δD values between -70‰ and -130‰). Darker colors represent the highest probability of origin.

parts per thousand (‰) deviation from a standard ($\delta^{13}\text{C}$: Pee Dee Belemnite, δD Vienna Standard Mean Ocean Water). For $\delta^{13}\text{C}$ analysis, red blood cells were freeze-dried and powdered (Hobson et al. 1997). Powdered red blood cells and whole claw samples were weighed ($350\text{ }\mu\text{g}$) into tin capsules. For δD analysis, feathers were cleaned with a dilute detergent and then a 2:1 chloroform:methanol solution following the methods of Paritte and Kelly (2009). Feather material from the distal end (140 to $160\text{ }\mu\text{g}$) of each sample was removed and wrapped in a silver capsule. Stable isotope ratios of both $\delta^{13}\text{C}$ and δD were determined with a ThermoFinnigan Delta V isotope ratio mass spectrometer connected to an elemental analyzer (hydrogen analyses: TC/EA, Thermo-Finnigan, Bremen, Germany; carbon analyses: CosTech elemental analyzer). For hydrogen isotope ratios, a comparative equilibrium approach with calibrated keratin standards was utilized to correct for uncontrolled isotope exchange between non-carbon-bound hydrogen in feathers and ambient water vapor (Wassenaar and Hobson 2003). As a result, values presented are non-exchangeable feather hydrogen only. Repeat analysis of carbon and

hydrogen isotope standards during the study yielded an external repeatability of $\pm 0.3\text{‰}$ and $\pm 1.1\text{‰}$, respectively. For hydrogen isotope analysis, intra- and inter- assay error (\pm SD) was 1.7‰ and 2.9‰ based on repeat analysis of the same feather ($n=7$), respectively.

Timing of Migration

To distinguish whether black-and-white warblers captured at the stopover site were early or late relative to conspecifics migrating to the same breeding area destination, I separately examined the range of capture dates for black-and-white warblers assigned to each breeding area. I divided each migration season (2008, 2009, 2010, 2011) into 3 equal time periods (early, middle, late) based on the range of capture dates for each black-and-white warbler breeding area (southeast and boreal forest) in a given year.

Migratory Condition

To determine the condition of a black-and-white warbler captured at the stopover site, I subtracted a size-specific fat free mass from actual body mass at capture (Ellegren 1992, Owen and Moore 2006). Briefly, I estimated a size-specific fat-free mass utilizing data from black-and-white warblers captured at Johnson's Bayou (1993-2007, $n= 684$). First, I grouped birds according to common wing chord classes (1mm increments), and then regressed body mass on fat score for each wing chord class. The intercept (equivalent to fat score = 0) provided an estimate of fat-free mass for the specific wing chord class. In a second analysis, fat-free mass was regressed on corresponding wing chord lengths and the resultant equation ($y=0.104x + 2.0086$) was used to estimate size-specific fat-free mass for each black-and-white warbler. Larger migratory condition values indicate birds with more fat reserves, and thus greater migratory condition.

Statistical Analysis

I constructed linear mixed models with a Gaussian distribution and identity link function using the R package ‘lme4’ (Bates and Maechler 2011) to examine variation in a bird’s over-winter habitat quality (measured via $\delta^{13}\text{C}$) and condition upon arrival at the stopover site (size-specific migratory condition). I modeled each response variable ($\delta^{13}\text{C}$ and migratory condition) as a function of timing of arrival at the stopover site (early, middle, late), breeding destination (southeast and boreal), and a bird’s age and sex. In addition, each model also included year as a random effect to control for potential yearly differences in $\delta^{13}\text{C}$ and migratory condition values. To evaluate the importance of each explanatory variable, candidate models included a fully specified global model with all main effects, a null model, and reduced forms of the general model including all possible combinations of each factor. I incorporated biologically meaningful second-order interactions in both the full general model and reduced forms. Biological meaningful second-order interactions included: breeding with timing, age, and sex, and timing with age and sex. I used Akaike’s information criterion (AIC) to rank, compare, and evaluate all candidate models. I present all models with $\Delta\text{AIC} \leq 2$ as possible competing models for each response variable (considered the subset of best models; Burnham and Anderson 2002), and also present the null model for assessing the relative explanatory power of the models under consideration. I performed statistical analyses in R version 3.0.1 (R Development Core Team 2013).

Results

I captured a total of 223 black-and-white warblers during the four-year study period. Mean arrival dates at the study site for warblers with the highest probability of

breeding in the southeast (n=88) and boreal forest (n=73) were 105.0 ± 1.13 (range: 88.23 ± 2.50 to 122.75 ± 4.35) and 119.1 ± 0.80 (range: 110 ± 3.29 to 130.5 ± 2.22), respectively (Table 3).

Table 3

Number of black-and-white warblers captured at the stopover site during the four-year study period (2008 to 2011) assigned to breeding area destinations in the southeastern U.S. and boreal forest. I examined the range of capture dates for black-and-white warblers assigned to each breeding area separately, dividing each migration season into 3 equal time periods (early, middle, late) based on the range of capture dates for each breeding area in a given year. Stable carbon isotope values were determined for a subset of the birds, indicated by the number in parentheses.

Migration Timing	Breeding Area Destination	
	Southeast	Boreal Forest
Early	28 (16)	34 (21)
Middle	35 (23)	22 (17)
Late	25 (18)	17 (14)

I found that black-and-white warblers arriving early to the stopover site compared to conspecifics migrating to the same breeding area destination had more depleted $\delta^{13}\text{C}$ values than warblers arriving later to the stopover site (Figure 6), suggesting that birds arriving early to the stopover site originated from higher-quality mesic over-winter habitat. Model selection also indicated warbler's migrating to breeding area destination in the southeastern U.S. and boreal forest differed in $\delta^{13}\text{C}$ values (Table 4). $\delta^{13}\text{C}$ values were positively correlated with timing of migration for warblers migrating to both breeding area destinations, however, warblers migrating to breeding area destinations in the boreal forest had overall more enriched $\delta^{13}\text{C}$ values (southeast $x = -24.42 \pm 0.21$, boreal forest $x = -23.94 \pm 0.14$). In addition, $\delta^{13}\text{C}$ values of females were more depleted than males (females $x = -24.78 \pm 0.20$, males $x = -23.65 \pm 0.14$).

Table 4

Linear Mixed Models examining the variation in over-winter habitat quality measured via stable carbon isotope values of red blood cells and energetic condition during stopover for black-and-white warblers captured during spring migration. Explanatory fixed variables examined for both models included: timing of migration relative to conspecifics migrating to the same breeding destination (Timing), southeast or boreal forest breeding area destinations (Breeding), condition of a bird at capture (Condition), age, and sex of the warbler. Year was included as a random factor in all models. Top competing models ($\Delta AIC_c < 2$) based on Akaike's information criterion adjusted for small sample sizes (AIC_c) and the null model are shown. In addition, w_i (Akaike weights) for each model is shown.

Model Description	ΔAIC_c^a	w_i
Over-winter Habitat Quality		
Timing, Sex	0.00	0.23
Timing, Breeding, Sex	0.80	0.15
Null	20.40	0.00
Migratory Condition		
Timing, Breeding	0.00	0.30
Timing, Breeding, Sex, Timing*Sex	0.10	0.17
Null	7.50	0.01

^a AIC of top model: over-winter habitat quality: -176.5, migratory condition: -138.1

The relationship between migratory condition and the timing of migration was more complex (Figure 7). Top candidate models indicated an interaction between the timing of migration and a bird's sex, suggesting that a bird's migratory condition during stopover was not only a function of the timing of migration, but also a bird's sex (Table 4). Contrary to my prediction, male black-and-white warblers arriving early to the stopover site compared to conspecifics migrating to the same breeding area destination

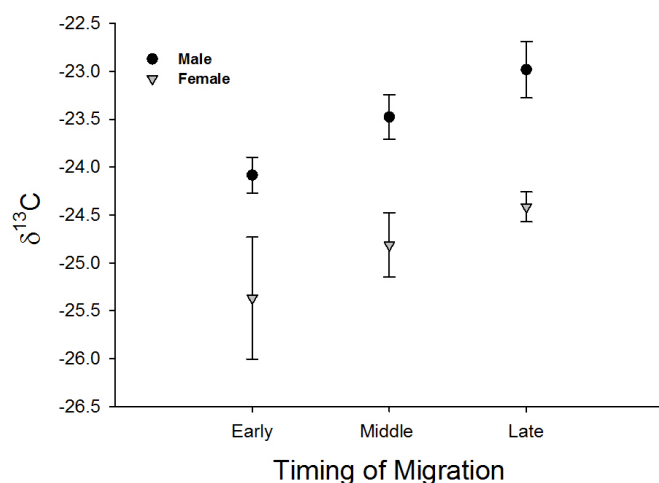


Figure 6. Average stable carbon isotope values (\pm SE) of red blood cells collected from black-and-white warblers captured during spring migration as a function of the timing of migration and a warbler's sex. Timing of migration was determined by dividing each migration season into 3 equal time periods (early, middle, late) based on the range of capture dates for each breeding area destination (southeast and boreal forest) in a given year. There was a positive relationship between stable carbon isotope values and the timing of migration for both males and females, indicating that warblers arriving earlier to the stopover site originated from higher-quality mesic habitat when compared to conspecifics migrating to the same breeding area destination.

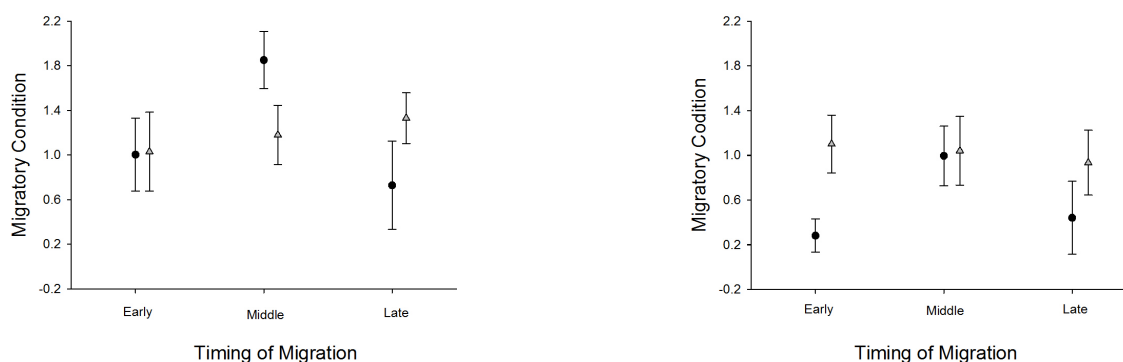


Figure 7. Average migratory condition values (\pm SE) as a function of the timing of migration for black-and-white warblers migrating to southeast (left panel) and boreal forest (right panel). Timing of migration was determined by dividing each migration season into 3 equal time periods (early, middle, late) based on the range of capture dates for each breeding area destination (southeast and boreal forest) in a given year. Male warblers (black circles) arriving early and late to the stopover site compared to

conspecifics migrating to the same breeding area destination have lower migratory condition compared to birds arriving during mid migration for each breeding area destination. However, there is no difference in migratory condition for female warblers (gray triangles) throughout the migration season.

had lower or equal migratory condition values as male warblers arriving late to the stopover site. However, female Black-and-white warblers did not differ in migratory condition across the migration time periods (Figure 7). Patterns of migratory condition for males and females across the migration time periods were the same for warblers migrating to breeding area destinations in the southeast and boreal forest. However, warblers migrating to breeding area destinations in the boreal forest had overall lower migratory condition values (southeast males: $x=1.30 \pm 0.20$, southeast females: $x=1.19 \pm 0.16$, boreal forest males: $x=0.52 \pm 0.13$, boreal forest females: $x=0.99 \pm 0.18$).

Discussion

This study demonstrates the power of integrating information from multiple phases of a migrant's annual cycle to assess the relative strength of carry-over effects between seasons. I found that the quality of over-winter habitat, inferred from $\delta^{13}\text{C}$ values, strongly influenced the timing of migration for black-and-white warblers when I accounted for differential timing of migration between breeding populations. Among birds migrating to the same breeding destination, males and females arriving early to the stopover site originated from higher-quality habitat than later arriving birds, suggesting that the benefits of occupying high-quality habitat during the over-winter time period carry-over to positively influence the timing of migration. The strong relationship I found between over-winter habitat quality and arrival timing at the stopover site is consistent with studies on the breeding grounds (males only; Marra et al. 1998, Norris et

al. 2004, Reudink et al. 2009, Tonra et al. 2011). However, carry-over effects of winter habitat quality on a bird's migratory condition was more complex, suggesting different migration strategies were employed by males depending on whether they were early or late compared to conspecifics migrating to the same breeding area destination. Moreover, males and females exhibited different patterns of migratory condition throughout the migration season.

While male warblers arriving early to the stopover site originated from higher-quality over-winter habitat, contrary to my expectation, they were not in better migratory condition than later arriving conspecifics that originated from poor-quality over-winter habitat. Studies examining habitat-specific differences in the quality of over-winter habitat have revealed that birds over-wintering in high-quality mesic habitats are in better condition during the dry season compared to birds over-wintering in poor-quality xeric habitats (Marra et al. 1998, Studds and Marra 2007, 2011, Thomas 2012), resulting in earlier fat deposition (Smith et al. 2010) and earlier departure for spring migration (Marra et al. 1998, Studds and Marra 2005, 2011). However, the influence of over-winter habitat occupancy on a bird's condition en route (Bearhop et al. 2004, González-Prieto and Hobson 2013, this study) and upon arrival at the breeding grounds (Marra et al. 1998, Norris et al. 2004, Tonra et al. 2011) has been varied. Black-throated blue warblers (*Setophaga caerulescens*) captured during migration at a stopover site within their wintering grounds found that birds from high-quality over-winter habitats (depleted $\delta^{13}\text{C}$ values) were in better migratory condition than birds from poor-quality over-winter habitat (enriched $\delta^{13}\text{C}$; Bearhop et al. 2004). However, migratory condition was related to over-winter habitat quality for only 3 out of 7 migratory species captured at a stopover

site near their breeding grounds in Manitoba, Canada (González-Prieto and Hobson 2013). Likewise, most studies of American redstarts on their breeding grounds in northern latitudes have not found a relationship between condition upon arrival and winter habitat quality (Norris et al. 2004, Tonra et al. 2011, but see Marra et al. 1998). The variable results among studies suggests that many factors potentially contribute to a bird's energetic condition, and that a migrant's energetic state at any given time is most likely a function of the cumulative effect of conditions and events encountered in the previous season as well as en route, and breeding destination.

A striking finding of this study was the consistency in the patterns of migratory condition across different time periods between the two breeding populations (southeastern U.S. and boreal forest in Canada), for both males and females, considering these populations stopover at the study site at different times. The reduced migratory condition of males arriving early to the stopover site compared to conspecifics migrating to the same breeding area destination is not a function of the time of season, but most likely a migrant's migration strategy. For example, strong selection pressures to arrive early on the breeding grounds to secure high-quality breeding territories (Møller 1994) with the ultimate pay-off of increased reproductive success (Saino et al. 2004, Norris et al. 2004, Smith and Moore 2005) may drive males from high-quality winter habitat to adopt a high-risk, high-reward strategy. Such that males arriving at the stopover site early minimize time at the expense of energy (Alerstam and Lindstrom 1990), resulting in a smaller margin of safety to buffer the effects of adverse weather or scarcity of food, increasing the risk of mortality during migration. In contrast, males arriving during mid migration regardless of breeding destination had the highest migratory condition and $\delta^{13}\text{C}$

values intermediate to early and late arriving warblers, a pattern consistent with a more conservative strategy of energy consumption. However, male warblers from poor-quality over-winter habitat that arrived late to the stopover site compared to conspecifics migrating to the same breeding destination were in poor migratory condition, suggesting that their condition en route was strongly influenced by occupancy of poor-quality over-winter habitat. The energetic cost of non-stop flight across the Gulf of Mexico, an ecological barrier that nearly all-eastern Neotropical migrants must negotiate (Rappole 1995), may exaggerate the consequences of different migratory strategies on migratory condition and the associated risks.

The stringencies of time pressures during migration most likely differ between males and females (Dierschke et al. 2004, Moore et al. 2003) given different selection pressures associated with breeding success. While the reproductive success of males is tightly linked to timing of arrival on the breeding grounds (Møller 1994, Marra et al. 1998, Norris et al. 2004, Smith and Moore 2005), females also increase their reproductive success through arrival at the breeding grounds with excess fat reserves (Smith and Moore 2003, Moore et al. 2005b). Therefore, I would expect females to adopt a strategy of minimization of energy consumption in order to arrive on the breeding grounds with excess fat reserves. Consistent with this idea, I found that the migratory condition of females was the same regardless of the timing of migration or breeding area destination, suggesting a similar migration strategy utilized by all black-and-white female warblers.

Whereas the specific winter origin of black-and-white warblers captured at the stopover site is not known, previous studies using δD found a strong connectivity between populations on the wintering and breeding grounds (Marra and Studds 2013).

Warblers migrating to the boreal forest most likely over-winter in Central America, and warblers migrating to the southeastern U.S. most likely over-winter in the eastern Caribbean Islands. Individuals over-wintering across large geographic areas could potentially have differences in $\delta^{13}\text{C}$ that is not necessarily associated with over-winter habitat quality given large-scale latitudinal variation in $\delta^{13}\text{C}$ owing to changes in the proportion of C3, C4, and CAM plants (Still et al. 2003). For example, birds over-wintering further south could have more enriched $\delta^{13}\text{C}$ values simply owing to latitudinal variation in $\delta^{13}\text{C}$ values. However, understanding patterns of migratory connectivity for black-and-white warblers and examining questions in the context of specific breeding populations that over-winter across a relatively small latitudinal gradient reduces the potential that differences in $\delta^{13}\text{C}$ values related to the timing of migration is a function of large-scale latitudinal variation in $\delta^{13}\text{C}$ values, but is a reflection of habitat-specific differences in $\delta^{13}\text{C}$ values. The pattern of more enriched $\delta^{13}\text{C}$ values for birds migrating to breeding area destinations in the boreal forest is consistent with latitudinal differences in C3 values between the most likely over-winter regions of the two breeding area destinations (i.e., warblers over-wintering in Central America would have more enriched $\delta^{13}\text{C}$ values than warblers over-wintering in the Caribbean Islands).

CHAPTER III
MIGRATORY CONNECTIVITY AND THE EN ROUTE MIGRATION STRATEGIES
OF MIGRATORY BIRDS

Abstract

I utilized stable hydrogen isotopes to geographically link individual black-and-white warblers (*Mniotilta varia*) captured during spring migration with breeding area destinations to understand how a migrant's stopover strategy (refueling rate and stopover duration) interacts with other periods of the annual cycle. In doing so, I was able to distinguish between birds with short and long distances remaining on migration and between birds that arrived early and late to the stopover site compared to conspecifics migrating to the same breeding area destination. I found that where a bird is in relation to its final breeding destination strongly influences the strategy a bird utilizes at a stopover site. Black-and-white warblers close to their breeding area destinations in the southeastern U.S. refueled at overall higher rates and stayed longer at the stopover site than warblers migrating longer distances to breeding area destinations in the boreal forest of Canada. Moreover, a black-and-white warbler's migration strategy was strongly influenced by the timing of arrival at the stopover site, which is largely driven by habitat conditions experienced in the previous phase of the annual cycle. I found considerable differences in refueling rates and duration of stopover among black-and-white warblers migrating short distances to breeding area destinations in the southeastern U.S. reflective of differences in migratory condition and timing of arrival at the stopover site. In contrast, migration strategies among warblers migrating to breeding area destinations in the boreal forest of Canada were consistent regardless of migratory condition or timing of

arrival at the stopover site, suggesting they primarily used the stopover site to rest after nonstop flight across the Gulf of Mexico before quickly resuming migration. This study demonstrates that I can begin to disentangle the complexity of migration when I examine factors known to be important to the success of migration in the context of other periods of the annual cycle. This is a critical step towards understanding population dynamics of migratory species, given that migration is the phase of the annual cycle most often thought to be limiting migratory birds.

Introduction

The seasonal movements of migratory organisms are controlled by processes that act across multiple spatial and temporal scales and play a major role in determining the fate of individuals as well as the structure and dynamics of the populations, communities and ecosystems they comprise (Nathan 2008). Large-scale seasonal movements expose migratory species to an array of differing environmental conditions such that the ecology, behavior, and life history strategies of migratory species must balance selection pressures associated with each phase of the annual cycle (Alerstam and Lindstrom 1990). Populations can be limited by events on the breeding grounds, non-breeding grounds, along migration routes, and through interactions between the different phases (Webster et al. 2002). Moreover, events or conditions in one season can produce residual effects that have consequences on an individual's success in subsequent periods of the annual cycle (Webster et al. 2002, Moore et al. 2005b, Harrison et al. 2010). Therefore, scientists increasingly realize that to understand the ecology of a migratory species in any one phase of the annual cycle requires an understanding of how other phases may interact with and influence the period of interest (Greenberg and Marra 2005). However,

establishing seasonal interactions between different phases of the annual cycle in geographically separate locations is one of the great research challenges of our time (Webster et al. 2002, Wilcove and Wikelski 2008).

Understanding of the physiology, behavior, and ecology of migratory birds during migration has been limited by the lack of knowledge of a bird's point of departure as well as their ultimate destination. Long-distance migration is energetically expensive, and the energy necessary for an entire migration typically exceeds the amount of fuel reserves that small passerines can store and carry (Blem 1990). Therefore, over seventy percent of migration is spent at stopover sites resting and replenishing energy reserves (Hedenstrom and Alerstam 1997). The negative fitness consequences associated with late arrival to the breeding grounds (Møller 1994, Smith and Moore 2003, Moore et al. 2005b, Reudink et al. 2009) should drive birds to minimize the overall time spent migrating (e.g., time-minimization *sensu* Alerstam and Lindstrom 1990). Hence, the rate at which birds gain body mass at a stopover site and the duration of stopover, two critical factors influencing the speed of migration, are decisive for a successful migration (Hedenstrom 2008). Because refueling rates and stopover decisions are critical for a successful migration, it is important to understand how these variables may differ among stopover sites, throughout the migration season, and between species. However, a migrant's stopover strategy (i.e., refueling rate and duration of stopover) at a given stopover site is not only a function of a bird's current energetic state, quality of stopover habitat, and weather conditions (Moore et al. 1995), it is also strongly influenced by the cumulative effect of conditions and events encountered in the previous season and en route as well as by the distance remaining to destination. Difficulty in determining a migrant's geographic linkage to

stationary phases (i.e., over-wintering and breeding areas) of the annual cycle and the conditions a migrant experiences prior to arrival at a stopover site hinders our ability to understand fundamental aspects of migration, the phase of the annual cycle thought to be limiting migratory birds (Sillett and Holmes 2002, Newton 2004, Moore et al. 2005a).

Recent advances in the use of intrinsic markers (e.g. stable isotopes, genetics) have increased our understanding of patterns of migratory connectivity between phases of the annual cycle. These markers have enhanced our ability to discern at broad scales how breeding populations are spatially distributed throughout the annual cycle (Hobson and Wassenaar 2008, Smith et al. 2005), providing critical information about the timing (Kelly et al. 2002b, Kelly 2006, Paxton et al. 2007) and overall distance of migration for different breeding populations (Clegg et al. 2003, Kelly et al. 2005, Boulet et al. 2006). In addition, studies using stable carbon isotopes ($\delta^{13}\text{C}$) have revealed seasonal interactions between the stationary periods, showing that the quality of over-winter habitat influences a bird's condition and timing of departure from over-winter sites (Marra et al. 1998, Strong and Sherry 2001, Studds and Marra 2005, 2007, 2011, Thomas 2012) with cascading impacts on future reproductive success (Marra et al. 1998, Norris et al. 2004, Reudink et al. 2009). Studies examining seasonal interactions have primarily focused on linking breeding and over-wintering phases (Marra et al. 1998, Saino et al. 2004, Reudink et al. 2009), while largely ignoring the intervening migratory time period (but see Bearhop et al. 2004, Moore et al. 2005b). However, the accumulation of studies documenting 1) migratory connectivity for a growing number of species and 2) the consequences of the quality of habitat during the non-breeding period on a migrant's over-winter condition, permits me to ask in-depth questions about stopover ecology

which were previously not possible. Previous studies using stable isotopes provide information on the spatial and temporal distribution of migratory birds during migration, enabling stronger inferences to be made about how stopover strategies may vary between populations. For example, I can now examine factors known to be important to the success of migration, such as refueling rates and stopover duration, in the context of conditions experienced prior to migration and a bird's breeding destination.

Understanding how migrants adjust migration strategies in order to optimize migration success is critical to understanding of population dynamics of migratory birds.

In this study, I investigated how a migrant's stopover strategy (refueling rate and stopover duration) interacts with other periods of the annual cycle. I utilized stable hydrogen isotopes (δD) to geographically link individual black-and-white warblers (*Mniotilta varia*) captured during spring stopover with breeding area destinations. In doing so, I determined (1) how far warblers must travel from the stopover site to their breeding area and (2) whether warblers were early or late relative to other birds traveling to the same breeding destination. Moreover, I have previously shown that the timing of arrival of black-and-white warblers at the stopover site is strongly influenced by carry-over effects of habitat occupancy during the over-winter time period (see Chapter II). For example, among birds migrating to the same breeding area destination, black-and-white warblers from poor-quality over-winter habitat, inferred from $\delta^{13}C$ values, arrive later to the stopover site and in poorer condition compared to earlier arriving warblers. Integrating information about an individual's breeding area destination and over-winter habitat quality allows for specific predictions about a migrant's stopover strategy. Specifically, warblers arriving late to the stopover site will refuel at a faster rate and

stopover for a shorter duration than conspecifics migrating to the same breeding destination in order to “catch-up” with the overall time-schedule of migration (*sensu* Alerstam and Lindstrom 1990). That said, the distance remaining to a breeding area destination may also interact with a migrant’s stopover strategy. Indirect evidence from band recovery data suggests that the speed of migration varies positively with migratory distance (e.g., Ellegren 1993, Fransson 1995), which is consistent with time-minimization (*sensu* Alerstam and Lindstrom 1990). Therefore, individuals migrating longer distances to more northern breeding locations will refuel faster and stopover for a shorter duration in order to attain a high speed of migration than individuals migrating to southern breeding destinations.

Methods

Study site and species

Black-and-white warblers were captured during spring migration 2008 to 2011 with mist-nets (12 x 2.6m, 30mm mesh) on a daily basis, 21 March to 13 May, for approximately 8 hrs (0800 to 1700) at a long-term migration banding station located along the northern coast of the Gulf of Mexico in Cameron Parish, Louisiana (29°45’N, 93°37’W). Black-and-white warblers do not over-winter or breed at the study site; so all warblers captured were transients. Nets were monitored every 30 minutes and birds were brought back to a central location for bleeding and banding. The time elapsed between extracting a bird from a net and blood sampling was recorded (bleedtime). All birds were bled within 20 minutes of capture, and blood samples were taken on average within 6.03 ± 0.34 minutes of extracting a warbler from a mist net. Upon capture, black-and-white warblers were banded with a USGS band and a unique color band combination and then a

blood sample via brachial vein puncture was taken for blood plasma metabolites (plasma) and $\delta^{13}\text{C}$ analysis (red blood cells; see Chapter II). After blood sampling, birds were aged (after-second year or second year) and sexed according to Pyle (1997), and then weighed to nearest 0.1g with an electronic scale. Additionally, two tail feathers (R5) were pulled for δD analysis. Blood samples were stored on ice, plasma separated, and then frozen at $-20\text{ }^{\circ}\text{C}$ within 6 hours of collection. In addition, color-banded black-and-white warblers were systematically searched for throughout the study site on a daily basis in 2009, 2010, and 2011.

Breeding Area Destination

Direct assignment to a geographic location utilizing only δD values from feathers is not reliable because of inter-individual differences in physiology, analytical error, and error associated with the isoscape to which birds are assigned (Wunder and Norris 2008). Therefore, I created a probabilistic surface of breeding area destinations for a range of δD values (southeast: -10‰ to -50‰ , boreal forest: -70‰ to -130‰) utilizing information from both the sample and error associated with δD values of feathers and precipitation (Hobson et al. 2009a, 2009b) to determine the probability that the breeding area of an individual black-and-white warbler captured at the migration station was from a particular breeding area destination (southeast or boreal forest). I adjusted stable feather isotope values (δD_f) of warblers captured at the stopover site to reflect stable hydrogen isotope values of precipitation (δD_p) utilizing the equation: $\delta\text{D}_f = 0.95 (\delta\text{D}_p) - 17.57$ (Hobson et al. 2012; non-ground foraging Nearctic-Neotropical migrants). To calculate an estimate of error I determined within-site standard deviations (s.d.) of δD values from fifty-five Wilson's warbler (*Cardellina pusilla*) and MacGillivray's warbler (*Geothlypis*

tolmiei) feather samples collected from 10 locations across their breeding ranges (data from Paxton et al. 2007). To visualize the likely point of origin for the ranges of δD values specified above, similar to Hobson et al. (2009a), I utilize Program R to generate a random sample of 1000 possible isoscapes values for each isotope value considered using a normal distribution with the isotope values as the mean and the average error (5.94 s.d.) generated from Paxton et al. (2007) above. A normal distribution was fit to the entire simulated dataset, and a probability of belonging to the chosen range of δD values was calculated for all deuterium values in the isoscape. Using the calculated probability densities I then reclassified the Bowen et al. (Bowen et al. 2005) isoscape using Spatial Analysis in ArcGIS v9.1 (ESRI, Redlands, CA) for each breeding area destination. Black-and-white warblers with δD values between -10‰ to -50‰ and -70‰ to -130‰ had the highest probability of breeding in the southeast and boreal forest (Figure 8), respectively, and thus only black-and-white warblers with δD values within these ranges were used in subsequent analyses.

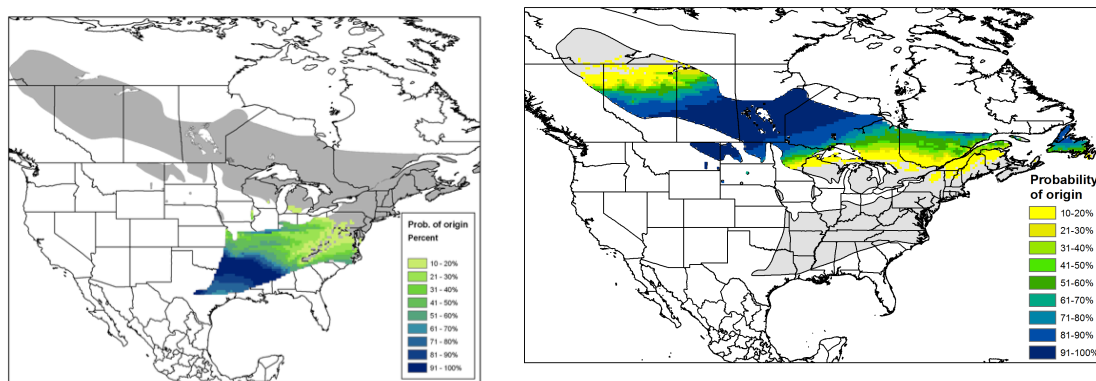


Figure 8. Black-and-white warbler breeding range showing the probability of breeding origin for the southeastern U.S. (left figure; δD values between -10‰ and -50‰) and boreal forest (right figure; δD values between -70‰ and -130‰). Darker colors represent the highest probability of origin.

Stable Isotope Analysis

Feathers were cleaned with a dilute detergent and then a 2:1 chloroform:methanol solution following the method of Paritte and Kelly (2009). Feather material from the distal end (140 to 160 ug) of each sample was removed and wrapped in a silver capsule. All stable isotope analyses were conducted at the University of Oklahoma utilizing a comparative equilibrium approach with calibrated keratin standards to correct for uncontrolled isotope exchange between non-carbon-bound hydrogen in feathers and ambient water vapor (Wassenaar and Hobson 2003). As a result, values presented here are non-exchangeable feather hydrogen only. Stable hydrogen isotope ratios for both feathers and keratin standards were determined on H₂ gases, produced by high-temperature flash pyrolysis of feathers using a high temperature (1450°C) pyrolysis elemental analyzer (TC/EA, ThermoFinnigan, Bremen, Germany) interfaced through an open split valve (Conflo III, ThermoFinnigan) with a ThermoFinnigan Delta V isotope ratio mass spectrometer. Repeat analysis of hydrogen isotope standards during the study yielded an external repeatability of $\pm 1.1\text{‰}$, respectively. Intra- and inter- assay error (\pm SD) was 1.7‰ and 2.9‰ based on repeat analysis of the same feather (n=7), respectively. Stable hydrogen isotope ratios (²H/¹H) are reported in delta (δ) notation, in per-mil units (‰), where $\delta D_{\text{sample}} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$, relative to a standard (Vienna standard mean ocean water [VSMOW]).

Timing of Migration

To distinguish whether black-and-white warblers captured at the stopover site were early or late relative to conspecifics migrating to the same breeding area destination, I separately examined the range of capture dates for black-and-white warblers assigned to

each breeding area. I divided each migration season (2008, 2009, 2010, 2011) into 3 equal time periods (early, middle, late) based on the range of capture dates for each black-and-white warbler breeding area (southeast and boreal forest) in a given year.

Migratory Condition

To determine the condition of a black-and-white warbler captured at the stopover site, I subtracted a size-specific fat free mass from actual body mass at capture (Ellegren 1992, Owen and Moore 2006). Briefly, I estimated a size-specific fat-free mass utilizing data from black-and-white warblers captured at Johnson's Bayou (1993-2007, n= 684). First, I grouped birds according to common wing chord classes (1mm increments), and then regressed body mass on fat score for each wing chord class. The intercept (equivalent to fat score = 0) provided an estimate of fat-free mass for the specific wing chord class. In a second analysis, fat-free mass was regressed on corresponding wing chord lengths and the resultant equation ($y=0.104x + 2.0086$) was used to estimate size-specific fat-free mass for each black-and-white warbler. Larger migratory condition values indicate birds with more fat reserves, and thus better migratory condition.

Measurement of Fuel Deposition Rate

Measurement of blood plasma metabolites are a robust method to assess the instantaneous refueling rate of an individual bird based on a single capture (Jenni-Eiermann and Jenni 1994, Schaub and Jenni 2001), and have been widely used to examine a migratory bird's energetic state during stopover (Guglielmo et al. 2005, Lyons et al. 2007, Seewagen et al. 2013). Plasma triglyceride levels increase during feeding and fat deposition, whereas β -hydroxy-butyrate levels increase during fasting and mass loss (Jenni-Eiermann and Jenni 1994). Prior to laboratory analysis, all plasma samples were

diluted two-fold in 0.9% NaCl solution so that concentrations of samples fell within the standard curve. Metabolites were assayed on a microplate spectrophotometer in 400 ml flat bottom microplates. Plasma triglyceride was measured sequentially by colorimetric endpoint assay (Sigma, St. Louis, MO: 5 μ l plasma, 240 μ l glycerol reagent, 60 μ l triglyceride reagent; (Guglielmo et al. 2002). β -hydroxy-butyrate was measured directly by kinetic endpoint assay (R-Biopharm, Marshall, MI, USA; (Guglielmo et al. 2005). For each assay, samples were analyzed in duplicate and values were averaged. All coefficients of variation between replicate samples were less than 15%.

Statistical Analysis

Metabolite concentrations were ($\log_{10} + 1$) transformed to satisfy assumptions of normality. A refueling index for each warbler was then calculated from the concentrations of transformed triglycerides and β -hydroxy-butyrate using the first principle component axis of a principle component analysis (Schaub and Jenni 2001). Larger refueling index values indicate higher refueling rates. However, the average of the refueling index scores is zero, hence negative scores do not reflect negative refueling rates.

I examined variation in refueling index values with a two-tiered modeling approach using R version 3.0.1 (R Development Core Team 2013). First, I selected *a priori* four methodological variables that have been shown to influence plasma metabolite levels for model inclusion (Guglielmo et al. 2002, 2005): time in minutes elapsed between extracting a bird from mist net and blood sampling (Bleedtime), time in minutes elapsed between sunrise and blood sampling (Daytime), the day of the year when the blood sample was collected (Ordinal date), and year blood sample was taken (Year). I

constructed general linear models (GLM) with Gaussian distributions and identity link functions with refueling index values as the response variable. I used Akaike's information criterion adjusted for small sample sizes (AICc; Burnham and Anderson 2002) to rank candidate models that included all possible combinations of each factor (n=14) and a null model. Variables contained in the top model were then included in the subsequent models.

In our second set of candidate models, I constructed GLM with a Gaussian distribution and identity link function to examine variation in refueling index values as a function of timing of arrival at the stopover site categorized as early, middle, or late compared to conspecifics migrating to the same breeding area destination (Timing) and a warbler's breeding area destination categorized as southeast or boreal forest (Breeding). In addition, I included a warbler's migratory condition (Condition), the age of the warbler categorized as second year or after-second year (Age), and sex of the warbler (Sex) as factors in the models given the importance of these variables in refueling rates during stopover (Moore et al. 1995). Methodological variables included from the previous analysis were bleedtime and year. To evaluate the importance of each explanatory variable, candidate models (n=169) included a fully specified global model with all main effects, a null model, and reduced forms of the general model including all possible combinations of each factor. I incorporated biologically meaningful second-order interactions in both the full general model and reduced forms. Biologically meaningful second-order interactions included: breeding with timing, age, and sex, and timing with age and sex. I used Akaike's information criterion adjusted for small sample sizes (AICc) to rank, compare, and evaluate all candidate models. I present all models with

$\Delta AICc \leq 2$ as possible competing models (considered the subset of best models; Burnham and Anderson 2002), and also present the null model for assessing the relative explanatory power of the models under consideration. I also estimated the relative importance of each variable (j) by calculating $w_+(j)$, where $w_+(j)$ is the sum of w_i (Akaike weights) across all models in the set in which variable j occurred (Burnham and Anderson 2002). Variables with strong support have cumulative Akaike weights near 1.

I used a mark-recapture framework to estimate stopover duration of black-and-white warblers (Schaub et al. 2001), using both recaptures and re-sights subsequent to initial capture. I used Program MARK (White and Burnham 1999) to produce daily survival estimates using a Cormack-Jolly-Seber model (CJS) only, versus a CJS and Pradel model (see Pradel et al. 2005). With an assumption of no mortality at the stopover site, these daily survival rates are equivalent to a daily stopover rate, with stopover duration calculated in the same manner as an expected lifespan (Schaub et al. 2001). I was not interested in changes in stopover duration over the season, and therefore collapsed the encounter history so that the initial capture of all birds was “day 1” and the total encounter history file was 9 periods, the longest span between detections of any one individual. I examined the relative effects of timing, breeding, age, sex, and condition had on the stopover duration using an information criteria framework with correction for small sample size ($AICc$). No re-sights were conducted in the first year of the study, and I used a year function (1st year vs all others) to account for a different detection probability in that first year. Parameter estimates from the top models were model averaged, and for each individual I calculated the expected stopover duration based on their breeding destination and migratory condition.

I performed all statistical analyses in R version 3.0.1 (R Development Core Team 2013), Jump version 10.0.0 (SAS Institute 2012), and MARK 7.1 (White et al. 1999).

Results

I captured a total of 223 black-and-white warblers during the four-year study period. Warblers with a high probability of breeding in the southeast (n=88) and boreal forest (n=73) migrated through the study site spanning on average 34.5 ± 5.56 days and 20.5 ± 2.18 days, respectively (Table 5).

Table 5

Number of black-and-white warblers captured at the stopover site during the four-year study period (2008 to 2011) assigned to breeding area destinations in the southeastern U.S. and boreal forest. I examined the range of capture dates for black-and-white warblers assigned to each breeding area separately, dividing each migration season into 3 equal time periods (early, middle, late) based on the range of capture dates for each breeding area in a given year. Triglyceride and β -hydroxy-butyrate concentrations were successfully assayed for a subset of the warblers, indicated by the number in the parentheses.

Migration Timing	Breeding Area Destination	
	Southeast	Boreal Forest
Early	28 (13)	34 (19)
Middle	35 (14)	22 (13)
Late	25 (17)	17 (13)

Plasma concentrations of triglycerides and β -hydroxy-butyrate were negatively correlated ($r=-0.36$, $n=89$, $p<0.004$). The first principal component axis explained 67.6% of the variation between the concentrations of the two metabolites (eigenvalue=1.35, $X^2=17.616$, $df=1.35$, $p<0.0001$). Triglyceride concentrations loaded positively and β -hydroxy-butyrate concentrations negatively on the first principle component axis. Thus,

the principle component score (refueling index) is positively correlated with refueling rates.

Model selection of methodological variables suggested that year and time between extracting a bird from the mist net and blood sampling (Bleedtime) most strongly influenced refueling index values (Table 6). Therefore, year and bleedtime were also included as explanatory variables in the set of candidate models examining overall variation in refueling index values.

Black-and-white warbler's refueling index values were strongly influenced by a warbler's breeding destination and the timing of arrival at the stopover site compared to conspecifics migrating to the same breeding destination (Table 6). However, model selection also indicated there was strong support for an interaction between timing of migration and breeding destination (Table 6), suggesting different migration strategies for refueling rates between warblers migrating to breeding areas in the southeastern U.S. and those migrating to boreal forest breeding areas in Canada (Figure 9). The higher refueling index values for warblers migrating to breeding destinations in the southeast (southeast: $x = 0.26 \pm 0.22$) were strongly driven by high refueling index values of warblers arriving early and late to the stopover site. In contrast, refueling index values were consistent among warblers migrating to the boreal forest (boreal forest: $x = -0.13 \pm 0.26$) regardless of when during migration they stopped over at the stopover site. There was also strong support for the influence of a warbler's condition at arrival at the stopover site and bleedtime on refueling index values (relative importance = 1.00 for both variables), such that refueling index values varied negatively with bleedtime and

positively with condition index values. In contrast, there was weak support for an influence of year on refueling index values (relative importance = 0.19).

Table 6

Summary of model results for variables influencing refueling index values (methodological and overall model results) and stopover duration of black-and-white warblers captured during spring migration. For overall refueling index and stopover duration models all top competing models ($\Delta AIC_c < 2$) based on Akaike's information criterion adjusted for small sample sizes (AIC_c) and the null model are shown. Only the top model and null model are shown for the methodological refueling index model. In addition, the w_i (Akaike weights) for each model is shown. Explanatory variables examined include: timing of migration relative to conspecifics migrating to the same breeding destination (Timing), southeastern U.S. or boreal forest breeding area destinations (Breeding), migratory condition of a bird at capture (Condition), age and sex of the warbler. For the overall refueling index model I also included: time elapsed between capture and blood sampling (Bleedtime), year of blood sampling (Year).

Model Description ^a	ΔAIC_c ^c	w_i
Refueling Index Methodological		
Year, Bleedtime	0.00	0.21
Null	10.36	0.00
Overall Refueling Index		
Bleedtime, Breeding, Timing, Condition, Breeding*Timing	0.00	0.18
Bleedtime, Breeding, Timing, Condition	0.53	0.14
Year, Bleedtime, Breeding, Timing, Condition	1.77	0.08
Null	18.60	0.00
Stopover Duration ^b		
Timing, Condition	0.00	0.27
Timing, Breeding, Condition, Age	0.86	0.18
Timing, Breeding, Condition	1.39	0.14
Breeding, Condition	1.56	0.13
Timing, Breeding, Condition, Age, Sex	1.61	0.12
Null	42.15	0.00

^a Refueling index methodological and overall refueling index model were calculated using program R. Stopover duration model results calculated with the program MARK.

^b Model variables only shown for survival $a(\phi)$. Detection probability (p) was the same for all top models $p(\text{breeding, timing, condition, year})$

^c AIC of top model: refueling index methodological: 276.79, overall refueling index: 268.55, stopover duration: 1952.80

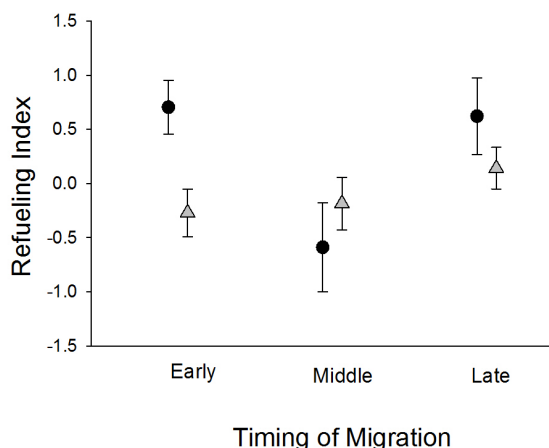


Figure 9. Average refueling index values for black-and-white warblers captured during spring migration 2008 to 2011 on the northern Gulf of Mexico at Johnson's Bayou, LA. The migration season was divided into 3 equal time periods (early, middle, late) based on the range of capture dates for each breeding area in a given year. Refueling index values were calculated for each warbler from the concentrations of $(\log_{10} + 1)$ transformed triglycerides and β -hydroxy-butyrate concentrations using the first principle component axis of a principle component analysis. Larger refueling index values indicate higher refueling rates. However, the average of the refueling index scores is zero, hence negative scores do not reflect negative refueling rates. Black circles and gray triangles represent warblers assigned to breeding area destinations in southeastern U.S. and boreal forest, respectively.

The duration of time that a warbler spent at the stopover site was strongly influenced by a bird's condition at the stopover site along with breeding area destination and the timing of arrival at the stopover site compared to conspecifics migrating to the same breeding destination (Table 6). There was a negative relationship between stopover duration and a bird's condition with the average stopover duration increasing by 0.36 days for each unit reduction in migratory condition. Black-and-white warblers migrating short distances to their breeding grounds in the southeast had longer durations of stopover than warblers migrating to more northern breeding destinations in the boreal forest (Table 6). However, examination of expected stopover duration estimates from modeled average parameter estimates of top models ($\Delta AICc < 2$) suggests that patterns of stopover duration

may differ across the migratory season for birds migrating to different breeding area destinations (Figure 10). Among conspecifics migrating to the same breeding destination, those arriving early and in poor migratory condition had the longest duration of stopover. There was moderate support for second-year warblers having longer stopover durations than after-second-year warblers, but little support that the sex of the warbler influenced stopover duration (Table 6).

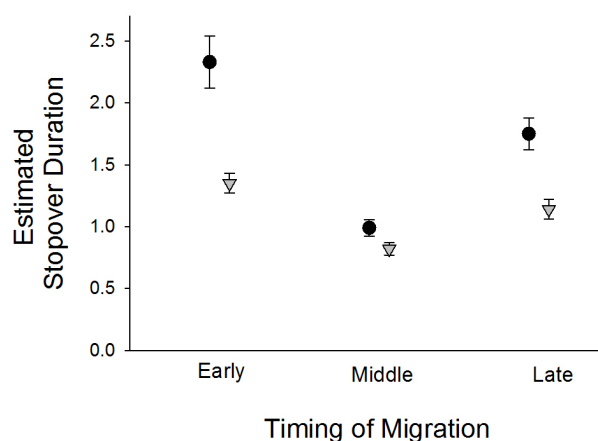


Figure 10. Expected stopover duration estimates from modeled average parameter estimates of top models ($\Delta AIC_c < 2$) for black-and-white warblers captured during spring migration. The migration season was divided into 3 equal time periods (early, middle, late) based on the range of capture dates for each breeding area in a given year. Black circles and gray triangles represent warblers assigned to breeding area destinations in southeastern U.S. and boreal forest, respectively.

Discussion

Migration is a complex system, and this study demonstrates the need to examine migration in the context of other periods of the annual cycle in order to understand the drivers of the system. By geographically linking an individual migrant captured during spring migration with a breeding area destination I was able to distinguish between birds with short and long distances remaining on migration, and between birds that arrived early and late to the stopover site compared to conspecifics migrating to the same

breeding area destination. A black-and-white warbler's migration strategy (refueling rate and stopover duration) was strongly influenced by both the timing of arrival at the stopover site, which is largely driven by habitat conditions experienced in the previous phase of the annual cycle (see Chapter II), and the distance remaining to a bird's breeding area destination.

Where a bird is in relation to its final breeding destination strongly influences the strategy a bird utilizes at a stopover site. Black-and-white warblers close to their breeding area destinations in the southeastern U.S. refueled at overall higher rates and stayed longer at the stopover site than warblers migrating longer distances to breeding area destinations in the boreal forest of Canada. Whereas re-gaining energy reserves is expected for all migrants after crossing an ecological barrier such as the Gulf of Mexico, our results suggest that as birds near their breeding destination they began to accumulate additional energy reserves in preparation for breeding (*sensu* Sandberg and Moore 1996). Black-and-white warblers migrating to breeding area destinations in the southeast not only had higher refueling rates and longer durations of stopover than warblers migrating to breeding destinations in the boreal forest, but they were in better overall migratory condition upon arrival at the stopover site (see Chapter II). While reproductive success is strongly tied to the timing of arrival on the breeding grounds, particularly for males (Møller 1994, Marra et al. 1998, Smith and Moore 2003, Norris et al. 2004), a bird's condition upon arrival to the breeding grounds also influences breeding success either directly or indirectly (Moore et al. 2005b). For example, excess fat reserves when arriving at the breeding grounds can benefit a bird by allowing it to sustain itself against inclement weather encountered during early spring on the breeding grounds (Møller

1994, Smith and Moore 2005), devote more time to territory or mate acquisition, accelerating the start of reproduction (Romero et al. 1997), or offset some of the energy needed for egg production (Winkler and Allen 1996). Gaining additional energy reserves prior to arrival on the breeding grounds can increase a bird's reproductive success (Smith and Moore 2003, Moore et al. 2005b). Therefore, given the importance of both time and condition on reproductive success, I would expect strong selection pressures to act on migratory behavior to optimize the speed of migration, a bird's migratory condition, or a combination of both (Alerstam and Lindstrom 1990), and that the selection pressures associated with energy and time may change according to a bird's time-programme (Jenni and Schaub 2003).

Further insight into migration strategies is gained when examining how refueling rates and stopover duration vary in relation to the timing of arrival to the stopover site, which is strongly influenced by carry-over effects from habitat occupancy during the over-winter time period. Other research at the same stopover site with black-and-white warblers (see Chapter II) indicates a strong relationship between over-winter habitat quality and arrival timing at the stopover site. Among birds migrating to the same breeding area destination, warblers arriving early to the stopover site originated from higher-quality over-winter habitat than later arriving conspecifics. However, males arriving both early and late to the stopover site were in poor migratory condition, regardless of breeding area destination. Reflective of differences in migratory condition and timing of arrival at the stopover site, I found considerable differences in refueling rates and duration of stopover among black-and-white warblers migrating to breeding area destinations in the southeastern U.S.. Birds originating from high-quality winter

habitat that arrived early to the stopover site where in poor migratory condition and had the longest duration of stopover and highest refueling rates, suggesting they were utilizing the stopover site to re-gain lost energy reserves prior to arrival at their breeding grounds. In contrast, birds arriving during mid migration compared to other birds migrating to breeding areas in the southeast arrived at the stopover site in good migratory condition. I found that birds within this time group primarily used the stopover site for resting before quickly resuming migration as indicated by low refueling rates and short duration of stopover. Last, birds arriving late to the stopover site that originated from poor-quality habitat as expected arrived at the stopover site in poor migratory condition. Late arriving birds exhibited a migration strategy similar to early arriving birds with high refueling rates to replenish lost energy reserves. However, birds arriving late had shorter durations of stopover than early arriving birds, presumably trying to catch-up with the overall time-schedule of migration.

In contrast, refueling rates and stopover durations were fairly consistent among black-and-white warblers migrating to breeding area destinations in the boreal forest of Canada. Regardless of migratory condition or timing of arrival at the stopover site, refueling rates were lower and durations of stopover shorter, suggesting that migrants destined for the boreal forest of Canada primarily used the stopover site to rest after nonstop flight across the Gulf of Mexico before quickly resuming migration. I predicted black-and-white warblers migrating to breeding destinations at northern latitudes would exhibit a time minimization strategy of high refueling rates and short stopover duration. Short stopover durations and minimization of energy reserves carried over long distances found in this study are consistent with this prediction.

A migrant's stopover strategy is not only a function of a bird's current energetic state, the quality of the stopover habitat, and weather conditions (Moore et al. 1995), but it is also strongly influenced by the cumulative effect of conditions and events encountered in the previous season as well as en route, and the remaining distance of migration. Despite the intuitive appeal of this argument, empirical evidence is lacking for small landbird migrants because it is difficult to determine a migrant's geographic linkage to stationary phases not to mention conditions experienced by a migrant prior to arrival at a stopover site. While recent advances in technology such as day-light level geolocators have revolutionized our ability to track small landbird migrants across the annual cycle (Stutchbury et al. 2009, Bairlein et al. 2012, Delmore et al. 2012) and document seasonal interactions (Stutchbury et al. 2010). Tracking devices are of limited use for studying how en route migration strategies interact with other stages of the annual cycle given the low probability of recapturing birds during migration to retrieve geolocator data. To my knowledge, this study is the first to link information from all periods of the annual cycle to understand the stopover biology for a small intercontinental migratory bird. This study demonstrates that we can begin to disentangle the complexity of migration when we examine factors known to be important to the success of migration, such as refueling rates and stopover duration, in the context of other periods of the annual cycle. This is a critical step towards understanding population dynamics of migratory species, given that migration is the phase of the annual cycle most often thought to be limiting migratory birds (Sillett and Holmes 2002, Newton 2004, Moore et al. 2005a).

CHAPTER IV

DIFFERENTIAL MIGRATORY TIMING OF WESTERN POPULATIONS OF
WILSON'S WARBLER (*CARDELLINA PUSILLA*) REVEALED BY
MITOCHONDRIAL DNA AND STABLE ISOTOPES

Abstract

Molecular markers and stable isotopes have provided important insights into the migratory connectivity of small landbirds. Research integrating these two methods has primarily focused on linking breeding and wintering sites, rather than focusing on timing of migratory movement of different breeding populations. I used mitochondrial DNA and isotopic markers to infer the timing of various breeding populations of migrating Wilson's warblers (*Cardellina pusilla*) moving through a migratory stopover site, demonstrating the value of multiple sources of information in estimating the origin of migrants. Using mixed-stock analysis, I found that early spring migrants sampled in southwestern Arizona were dominated by warblers migrating to the West Coast of the contiguous United States, whereas later migrants included a large pulse of birds migrating to Alaska and western Canadian provinces. Stable hydrogen isotope data from individual birds showed the same timing pattern as genetic data. Had I used stable isotopes alone, I would not have been able to infer whether birds later in the migration season were most likely migrating to Alaska or the Interior West, given the large overlap in isotope values between those regions. The lack of mitochondrial group 2, common in the Interior West, in late-season migrants strongly suggests that these birds were migrating to breeding areas in Alaska or other northern regions. Studies that reveal the timing of migration of different breeding populations through stopover sites lay the

foundation for more in-depth examination of seasonal interactions between migration and the stationary phases of the annual cycle.

Introduction

Knowledge of migration routes and timing of movement between breeding and nonbreeding regions is essential for a full understanding of the ecology, evolution, and conservation of migratory species (Webster et al. 2002, Smith et al. 2005, Irwin et al. 2011, Ryder et al. 2011, Wilson et al. 2011), yet relatively little is known about patterns of migratory connectivity within most species of birds. Analyses of morphological variation (e.g., subspecies differences) and band recoveries have revealed some patterns of migratory connectivity over the past century, but only in species with strong geographic variation in morphology or a high recovery rate of banded birds (Ramos and Warner 1980, Bell 1997, Bairlein 2001). Moreover, the large geographic area over which migrants move during migration has limited the use of these methods in identifying migration routes and timing of migration for specific populations. Yet population trajectories for many migratory species vary across the breeding range (North American Bird Conservation Initiative Canada 2012), underscoring the need for knowledge of migratory connectivity between migration and stationary phases of the annual cycle.

During the past two decades, our ability to discern patterns of within-species migratory connectivity has dramatically increased with the use of intrinsic markers such as isotopes and genetic markers (Webster et al. 2002, Veen 2013). Genetic and isotopic analyses to date have revealed different components of geographic variation in Nearctic–Neotropical migrants. Molecular markers have primarily revealed strong genetic differences between eastern and western populations, with less structure within groups

(Smith et al. 2005). By contrast, the latitudinal gradient in hydrogen isotope ratios across North America (Bowen et al. 2005) has allowed for inferences to be made regarding movement of breeding populations along a north–south gradient (Hobson and Wassenaar 2008). However, the level of resolution is limited for each individual marker, which results in large uncertainty of migratory connectivity for most species. The integration of genetic and isotope markers allows finer-scale geographic resolution (Clegg et al. 2003, Boulet et al. 2006, Rundel et al. 2013).

Most research on migratory connectivity has focused on linking wintering and breeding populations (e.g., Clegg et al. 2003, Irwin et al. 2011, Rundel et al. 2013), with comparatively little attention given to inferring the routes and migration timing of birds actually sampled during migration itself (but see Kelly et al. 2002b, Kelly 2006, Paxton et al. 2007). Here, I utilize both genetic markers and stable isotopes to better understand which breeding populations of Wilson’s warbler (*Cardellina pusilla*) are moving through a stopover site during their northward migration, as well as the relative timing of movement of those populations. The extensive background on patterns of variation in genetic markers and stable hydrogen isotopes for Wilson’s warblers (Kelly et al. 2002b, Kimura et al. 2002, Clegg et al. 2003, Smith et al. 2005, Paxton et al. 2007, Irwin et al. 2011, Rundel et al. 2013) allows me to build upon results from past studies to enhance our understanding of migratory connectivity. Previous studies of molecular variation have shown strong differentiation between western and eastern breeding regions of Wilson’s warblers, specifically in terms of mitochondrial DNA (mtDNA; Kimura et al. 2002), up to nine microsatellites (Clegg et al. 2003, Rundel et al. 2013), and 257 nuclear DNA markers (AFLPs; Irwin et al. 2011), with less structure within regions than

between. Here, I show that by focusing on inferring the breeding regions of groups of birds (rather than specific individuals), mtDNA variation is much more informative than previously thought (Kimura et al. 2002, Clegg et al. 2003) in inferring patterns of migratory connectivity within the western breeding group.

First, I reexamined genetic structure within western breeding populations of Wilson's warblers, using mtDNA data presented by Kimura et al. (2002), and designed a method for assignment of warblers to a variety of mitochondrial haplotype groups. I then determined mitochondrial group membership and feather hydrogen isotope values of Wilson's warblers sampled during spring migration at a stopover site in southwestern Arizona, with the goal of inferring breeding regions of groups of individuals moving through at different times of the season. Specifically, I examined how mitochondrial haplotype groups changed across the migratory season and corresponded to changes in stable hydrogen isotope values collected from the same individuals. Previous studies with stable isotopes have indicated that Wilson's warblers have a "leap-frog migration" in which the more northern breeders migrate southward earlier in the fall, winter farther south, and migrate northward later in the spring (Kelly et al. 2002b, Clegg et al. 2003, Paxton et al. 2007). However, precise assignment of birds to particular breeding regions in western North America on the basis of stable hydrogen isotopes alone has been confounded by the influence of elevation and other geographic factors on stable hydrogen isotope values (Ingraham 1998). This study illustrates that genetic and isotopic analysis can be useful in understanding the movements of various breeding groups through migratory stopover sites, enhancing our ability to understand movements during the full annual cycle of migratory birds.

Methods

Sampling at stopover site

Wilson's warblers were sampled during spring migration at Cibola National Wildlife Refuge (NWR; 33°18'N, 114°41'W; elevation 60 m) in La Paz County, Arizona. Located on the main branch of the lower Colorado River, Cibola NWR is composed of narrow fragmented strips of riparian vegetation adjacent to the river corridor, surrounded by desert upland and agricultural fields (Anderson et al. 2004, Paxton et al. 2008). I passively sampled warblers with mist nets daily between 17 March and 16 May in 2008 and 2009. I banded all warblers captured with a federal aluminum bird band and recorded standard morphological measurements. For stable isotope analysis I pulled two rectrices (R4 on each side) and stored feathers in labeled, sealed envelopes until analysis. Wilson's warblers molt their feathers on their breeding grounds prior to fall migration (Kelly et al. 2002b, Paxton et al. 2007); thus, isotope characteristics of a bird's feather collected during spring migration should reflect that bird's previous breeding region. For genetic analysis, I took a blood sample via the brachial vein and mixed a drop of whole blood in Queen's lysis buffer (0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA, 1% *n*-lauroylsarcosine, pH 7.5; Seutin et al. 1991). Samples were stored on ice and then frozen within 6 hours of collection. Later, in the laboratory, DNA was extracted using standard phenol-chloroform extraction and diluted to a working concentration of 25 ng/μL.

Mitochondrial typing

I closely examined warbler mitochondrial control-region sequences (343 base pairs [bp]) that were originally generated and summarized by Kimura et al. (2002). Sequences were sent directly by Mari Kimura, who has attested to their validity. I

identified three restriction enzymes (*BsaI*: zero or one cut site; *MspI*: zero or one cut site; and *StyD4I*: 2 or 3 cut sites) that could be used in combination to distinguish major groups of haplotypes (Figure 11, Table 7). The relative frequencies of these haplotype digestion groups differ strongly between different sampling regions (Table 8).

Table 7

Summary of restriction enzymes and banding patterns used to assign Wilson's warblers to mitochondrial restriction digest groups.

Restriction enzyme	<i>BsaI</i>	<i>MspI</i>	<i>StyD4I</i>
	Two bands around 200 bp? (in addition to 400)	Bands at 300 and 100? (in addition to 400)	Bands just above and below 100? (in addition to 200)
Group 1	Yes	No	No
Group 2	No	No	Yes
Group 3	Yes	Yes	Yes
Group 4A	Yes	Yes	No
Group 4b	No	Yes	No
Group 5	No	No	No
Group 6	No	Yes	Yes
Group 7	Yes	No	Yes

Table 8

Mitochondrial digest groups names, digestion patterns (see Table 7 and Figure 11), and frequencies among 13 sampled breeding sites grouped into four broad regions, inferred by analysis of sequences and haplotype distributions generated by Kimura et al. (2002).

Digest Group	1	2	3	4A	4b	5	6	7	Total
Digest pattern (BsaI, MspI, StyD4I)	ynn	nnn	yyy	yyn	nyn	nnn	nyy	yny	
Boreal Forest									
Denali National Park, Alaska	3	0	3	9	0	0	0	0	15
Hinton, Alberta	6	0	2	3	1	1	0	0	13
Interior West									
Umatilla National Forest, Oregon	0	3	1	0	1	0	0	0	5
Tahoe National Forest, California	0	10	0	2	3	0	0	0	15
Kings Canyon National Park, California	0	7	0	2	3	0	0	0	12
Grand Mesa, Colorado	0	11	0	0	0	1	0	2	14
West Coast									
Mt. Baker National Forest, Washington	1	1	1	2	7	0	0	0	12
Suislaw National Forest, Oregon	0	0	0	1	14	0	1	0	16
Pillar Point California	0	0	0	5	12	0	0	0	17
Big Sur, California	1	0	0	5	2	0	0	0	8
East									
Camo Myrica, Quebec	0	0	0	0	0	16	0	0	16
Fredericton, New Brunswick	0	0	0	0	0	4	0	0	4
Hilliardton, Ontario	0	0	0	0	0	4	0	0	4

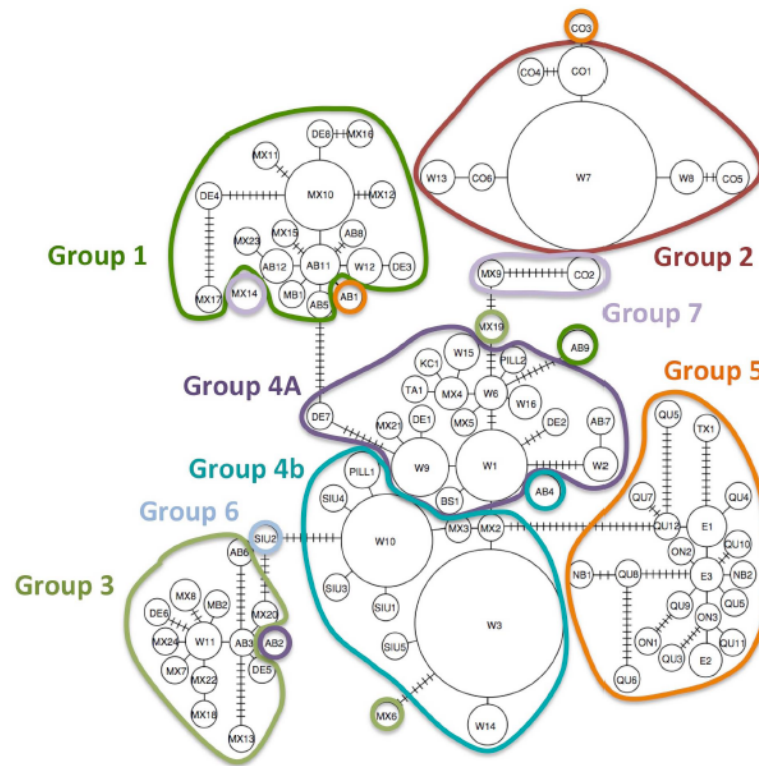


Figure 11. Wilson's Warbler mitochondrial haplotype network presented by Kimura et al. (2002; their figure 2), overlaid with restriction-digest group memberships (Table 7).

To amplify the control region, I used primers MT_H417 (5'-CGG TTC TCG TGA GAA GCG C- 3'), designed by Darren Irwin's lab, and LGL2 (5'- GGC CAC ATC AGA CAG TCC AT- 3') (Milá et al. 2007). Each 20- μ L reaction consisted of 1.5 μ L of template DNA (at 25 ng/ μ L), 2 μ L of 10X polymerase chain reaction (PCR) Buffer (Invitrogen, Carlsbad, California), 2.5 mM $MgCl_2$ (Invitrogen), 0.25 mM dNTPs (New England Biolabs [NEB], Ipswich, Massachusetts), 1.76 μ L of each primer (at 10 μ M), and 0.5 U of Taq polymerase (NEB). The thermal cycling protocol was 95°C for 3 min, 42°C for 60 s, 72°C for 60 s, followed by 10 cycles of 94°C for 60 s, 42°C for 60 s, 72°C for 60 s, followed by 32 cycles of 92°C for 60 s, 40°C for 45 s, 72°C for 45 s, followed by 72°C for 5 minutes. To ensure proper amplification, 5 μ L of each product was run for

12 min on a 2% agarose gel in 0.5X TAE, along with 1 μ L of 100 bp DNA size standard in a lane for comparison. The amplified product (376 bp, including primer sequences) appeared as a band next to the 400-bp size standard.

I divided each amplified product into three 5- μ L aliquots, each of which was digested with a different restriction enzyme. In the first, I added 0.11 μ L *BsaI* (10,000 U/mL; NEB), 0.45 μ L of NEB Buffer 4, and 3.44 μ L ddH₂O. In the second, 0.11 μ L *MspI* (20,000 U/mL; NEB), 0.45 μ L of NEB Buffer 4, and 3.5 μ L ddH₂O were added. In the third, I added 0.11 μ L *StyD4I* (10,000 U/mL; NEB), 0.45 μ L of NEB Buffer 4, and 3.44 μ L ddH₂O. Each digestion was then incubated for 2 hours at 37°C, followed by 15 min at 70°C to terminate the reaction. All 9 μ L of each digestion product was then run on a 3% agarose gel in 0.5X TBE, along with 1 μ L of 100 bp DNA size standard in a neighboring lane.

In the *BsaI* digest, some individuals have a cut site (recognition sequence and cut sites: 5'-GGTCTCN/-3' on one strand, and 3'-CCAGAGNNNNN/-5' on the other, where “/” indicates the cut site) and others do not, due to a C-T single nucleotide polymorphism (SNP) located 189 bp from the MT_H417 end of the fragment. This results in some digests that produce two fragments of length 190/194 bp (the two numbers indicating the length of the two strands of DNA, due to the enzyme cutting at different locations on the two strands) and 186/182 bp. These two fragments tend to appear as a single band on the agarose gel, just below the 200-bp size standard, indicating the presence of the C variant at this SNP. The absence of a band near the 200-bp size standard indicated the T variant.

In the *MspI* digest, only some individuals have a cut site (5'-C/CGG-3' on one strand, and 3'-GGC/C-5' on the other) due to a G-A-T polymorphism located 278 bp from

the MT_H417 end of the fragment. Thus, some digests have two fragments of length 275/277 bp and 101/99 bp. The presence of bands near the 300 and 100 size standards indicates the G variant at this SNP, whereas the absence of those bands indicates the A or T variants.

Finally, in the *StyD4I* digest, only some individuals have a cut site (5'-/CCNGG-3' on one strand, 3'-GGNCC/-5' on the other) located 259–263 bp from the MT_H417 end. Lack of that cut site is usually due to C-T-G polymorphism at 259 bp, although G-A polymorphisms were also observed at 262 bp and 263 bp (with the A variant observed in only one haplotype each). Cutting at that site results in a 118/113 bp fragment spanning from the LGL2 end, and the presence of a fragment just above the 100 bp size standard was taken to indicate the presence of this cut site. Most individuals also had another *StyD4I* cut site located 180–184 bp from the MT_H417 end of the fragment, resulting in a band of length 179/184 bp from the MT_H417 end, as well as bands of either 79/79 bp or 197/192 bp in the other direction (depending on whether the cut site above is present). Only the presence of the band just above 100 bp in size was diagnosed, because analysis of the sequences along with haplotype distribution data provided by Kimura et al. (2002) indicate that the cut site associated with that band was most geographically informative.

I decided to limit our analysis to the largest age and sex class, after-second-year (ASY) males, given the limits of time and budget. I focused on the samples collected in 2009, determining mitochondrial digest groups of 185 individuals. I also determined the mitochondrial groups of 62 individuals collected in 2008, so that I could compare patterns between years. Note that the 2008 samples to be genotyped were chosen with an intentional bias toward those with high or low isotopic ratios—hence, their haplotype

distributions should not be viewed as a random sample.

Mixed-stock analysis

I used a mixed-stock analysis to estimate the breeding destination of groups of migrating warblers on the basis of shared mtDNA haplotypes. I divided the warbler's breeding range into four regions (Boreal Forest, West Coast, Interior West, and East; Table 8) and divided the 2009 migratory season into weekly intervals. A mixed-stocked analysis estimates the frequency of mitochondrial haplotype groups for each breeding region based on samples collected across the warbler's breeding range (Table 8). Then, for each weekly group of migrants, the mixed-stock analysis determines the likelihood that observed mitochondrial haplotype groups within a group of migrants were contributed from each of the four defined breeding populations (Fournier et al. 1984). I emphasize that the mixed-stock analysis does not assign individual birds to breeding regions; rather, it provides maximum-likelihood estimates of the contribution of various breeding regions to a group of migrants, by comparing haplotype frequencies within the group with those in each breeding region. The greater the genetic differences among breeding groups, the stronger the inference, but estimates based on mixed-stock analysis are typically associated with large confidence intervals (Reynolds and Templin 2004). Mixed-stock analysis has been used widely in fisheries studies (Bolker et al. 2003, Okuyama and Bolker 2005) and occasionally in bird studies (Pearce et al. 2000, Paxton et al. 2011). I used a constrained maximum-likelihood approach with the mixed-stock package in program R (Bolker 2012).

Isotopes

Feathers were cleaned with a dilute detergent and then a 2:1 chloroform:methanol

solution, following the method of Paritte and Kelly (2009). Feather material from the distal end (140 to 160 μg) of each sample was removed and wrapped in a silver capsule. All stable isotope analyses were conducted at the University of Oklahoma, using a comparative equilibrium approach with calibrated keratin standards to correct for uncontrolled isotope exchange between non-carbon-bound hydrogen in feathers and ambient water vapor (Wassenaar and Hobson 2003). As a result, values presented here are non-exchangeable feather hydrogen only. Stable hydrogen isotope ratios for both feathers and keratin standards were determined on H_2 gases, produced by high-temperature flash pyrolysis of feathers using a high temperature ($1,450^\circ\text{C}$) pyrolysis elemental analyzer (TC/EA; ThermoFinnigan, Bremen, Germany) interfaced through an open split valve (Conflo III; ThermoFinnigan) with a ThermoFinnigan Delta V isotope ratio mass spectrometer. Repeat analysis of hydrogen isotope standards during the study yielded an external repeatability of $\pm 2.1\text{‰}$. Intra- and inter- assay error (\pm SD) were 2.2‰ and 3.6‰, respectively, based on repeat analysis of the same feather ($n = 13$). Stable hydrogen isotope ratios ($^2\text{H}/^1\text{H}$) are reported in delta (δ) notation, in per-mil units (‰), where $\delta\text{D}_{\text{sample}} = [(\text{R}_{\text{sample}}/\text{R}_{\text{standard}}) - 1] \times 1,000$, in relation to a standard (Vienna standard mean ocean water [VSMOW]).

To examine how feather isotope values corresponded to latitudinal gradients in precipitation across western North America, I adjusted stable feather isotope values (δD_f) to reflect stable hydrogen isotope values of precipitation (δD_p) using the equation $\delta\text{D}_f = 1.06 (\delta\text{D}_p) - 24.24$. The equation was generated using feather isotope values of breeding Wilson's warblers from Paxton et al. (2007; their table 1) and stable hydrogen isotope values of precipitation from Bowen et al. (2005). Although statistical approaches have

been designed to reduce uncertainty when mapping the origin of animals using stable hydrogen isotopes (Wunder and Norris 2008), our goal was not to directly map the origins of migratory birds, but to examine how broad-scale patterns of stable isotopes complement genetic data.

Results

I successfully designed a relatively quick and inexpensive method, using PCR amplification followed by restriction digests, to assign individuals to major mitochondrial groups (Figure 11). The geographic distributions (Figure 12) of these mitochondrial groups documented by Kimura et al. (2002) could then be used to estimate the breeding destination of weekly samples of birds on migration.

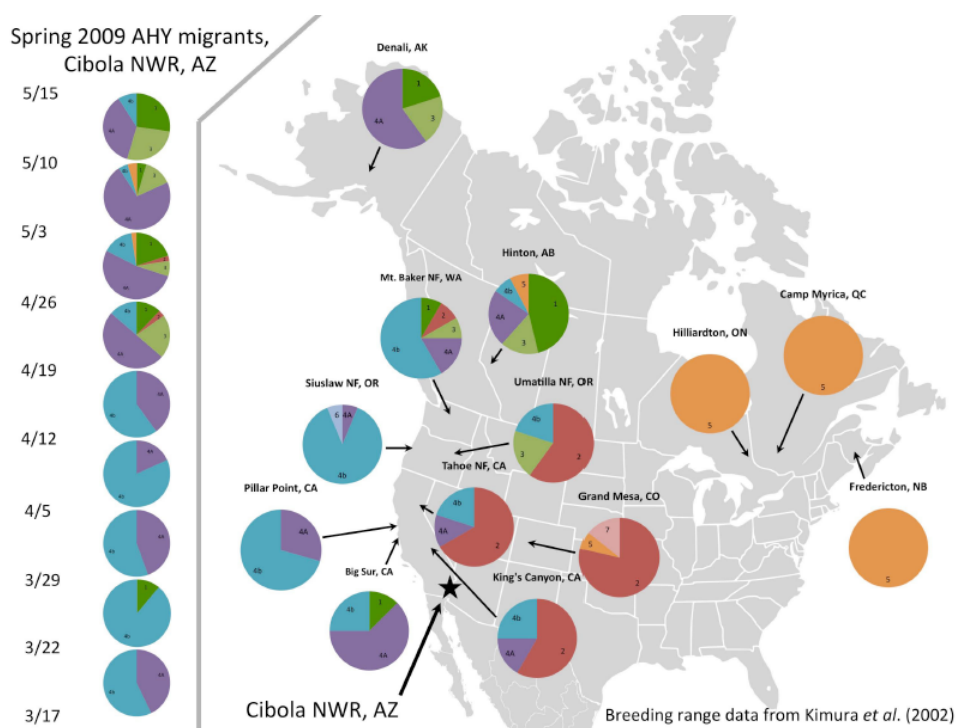


Figure 12. Map of North America showing mitochondrial digest-group distribution at sampling sites (Table 8; based on data from Kimura et al. 2002) along with (left) weekly digest group distributions of spring 2009 migrants captured at Cibola National Wildlife Refuge, Arizona.

Mitochondrial restriction-group membership of after-second year male Wilson's warblers migrating through southwestern Arizona differed dramatically across the 2009 migration season, and in a similar way across the 2008 migration season. The mixed-stock analysis estimated that warblers migrating early in spring 2009 were dominated by breeding populations along the California, Oregon, and Washington coast (Figure 12 and 13). In these early migrants, mitochondrial group 4b was most common, with 4A also at fairly high frequency (Figure 12), consistent with the frequency distributions of mitochondrial groups 4b and 4A within the West Coast breeding group (66% and 25%, respectively; Table 8). The mitochondrial-group distribution of migrants shifted dramatically at about the halfway point of the migration season, on about 19 April, with group 4b decreasing in frequency and groups 1, 3, and 4A increasing in frequency (Figure 12). The shift in the distribution of mitochondrial groups was reflected in the mixed-stock analysis, with the frequency of coastal breeding populations inferred to be migrating through the stopover site dramatically decreasing and populations breeding in the boreal forest in Alaska and Alberta increasing (Figure 13). Altogether, the results suggest a midseason shift between birds migrating primarily to coastal California, Oregon, and Washington and those migrating farther north to western Canada and Alaska.

The dominant mitochondrial group of warblers breeding in the Interior West group (e.g., Colorado, eastern California, and eastern Oregon) was mitochondrial group 2 (Table 8). For migrants captured in 2009, this group was found in only three individuals, which suggests that Interior West breeding populations are surprisingly rare migrants through southwestern Arizona (Figure 12 and 13). Mitochondrial group 5, which is at

very high frequency in eastern Canadian populations, was also very rare in the migratory samples, indicating that very few (if any) eastern birds migrate through the stopover site (Figure 12 and 13). It should be noted that there are two rare haplotypes in Kimura et al.'s (2002) breeding-season data that have the same group-5 digestion pattern as the eastern mitochondrial group but are, in fact, related to western haplotypes in group 1 or 2 (Figure 11); hence, the three group-5 birds in this study may not be eastern individuals.

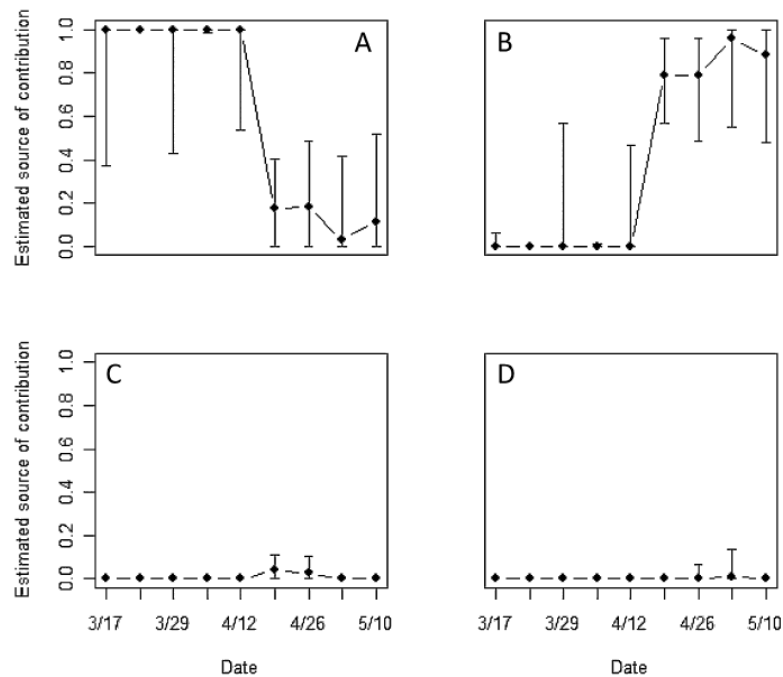


Figure 13. Mixed-stock analysis showing the estimated contributions of the four Wilson's Warbler breeding areas, (A) West Coast, (B) Boreal Forest, (C) Interior West, and (D) East, to weekly groups of Wilson's Warblers captured during spring migration in southwestern Arizona. Bars represent 95% confidence intervals.

Consideration of stable hydrogen isotope data supports and further clarifies these patterns (Figure 14). Consistent with the mixed-stock analysis and mitochondrial group composition (4A and 4b), isotopic ratios early in the migration season tended to be more enriched (less negative isotope values), most consistent with a breeding destination along

coastal California. Isotopic ratios declined gradually during the first half of the migration season, consistent with gradually more northern coastal breeding populations (e.g., Oregon) migrating through the stopover site. A fairly sudden shift in isotopic signatures, with a large pulse of birds with more depleted isotopic ratios, coincided with large increases in the frequencies of mitochondrial groups 1, 3, and 4A and a large decrease in the proportion of group 4b. Moreover, birds of mitochondrial groups 3 had remarkably depleted isotopic ratios, lower than all of the isotopic ratios from the first half of the season.

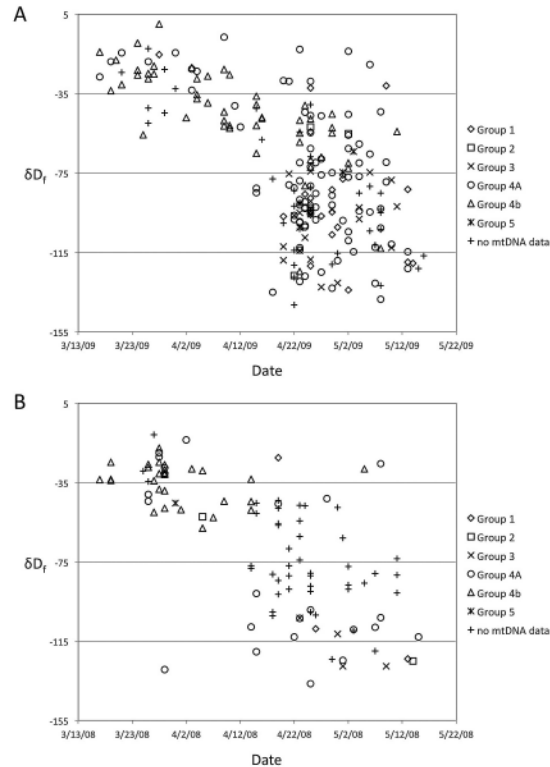


Figure 14. Plot of capture dates and hydrogen isotope ratios of (A) spring 2009 and (B) spring 2008 after-second-year male Wilson's Warblers migrating through Cibola National Wildlife Refuge, Arizona. Each symbol represents an individual bird, with different symbols representing different mitochondrial digest groups (see Figure 1, and Tables 1 and 2). Note that numerous samples in 2008 were not genotyped, and the genotyped ones were not chosen randomly.

Discussion

The use of genetic markers to draw inferences about migratory connectivity of small Nearctic–Neotropic landbirds has been most useful in differentiating large-scale patterns between eastern and western populations (Kimura et al. 2002, Clegg et al. 2003, Smith et al. 2005, Irwin et al. 2011, Rundel et al. 2013). Here, I have shown that variation in mtDNA can be used to infer the likely breeding regions of groups of migrating Wilson’s warblers within western regions of North America. Early spring migrants sampled in southwestern Arizona are primarily migrating to southern West Coast regions, whereas migrants later in the season include a large pulse of birds migrating much farther north, to Alaska and western Canada. I can also infer with high confidence that very few, if any, of the warblers captured at our site are migrating to breeding regions in the Interior West of the United States (e.g., eastern California, eastern Oregon, and Colorado) or the northeastern part of the continent (e.g., Ontario, Quebec, New Brunswick).

The results of the molecular genetic analysis show that geographic structuring in the frequencies of mitochondrial groups enables fairly strong conclusions regarding the likely breeding destination of groups of migrants moving through a stopover site. However, these conclusions are made with respect to populations of migrants, not individual birds. As Kimura et al. (2002) noted, the sharing of mitochondrial haplotypes among western populations precludes assignment of individual birds to breeding regions with a high degree of confidence. Although I cannot assign origin to individuals, the frequencies of haplotypes in a group of migrants can be used to estimate the probability that they belong to one or more breeding populations. For example, a single individual with mitochondrial group 4A sampled at the stopover site early in the migration season

could only be assigned to a very broad breeding region, including coastal and interior California, coastal Oregon and Washington, the Canadian Rockies, and Alaska, because group 4A is common in all those regions. However, a group of early-season individuals consisting of roughly half of group 4A and half of group 4b can with more confidence be assigned to breeding destinations within a much smaller area, namely the West Coast of the contiguous United States. The lack of mitochondrial groups 2, 3, 1, and 5 in the group of early-season migrants excludes the other breeding regions for the majority of the group. Although mixed-stock analysis sometimes has large uncertainty in estimates (Reynolds and Templin 2004), the results in this study suggest a large shift in source populations over a short period, resulting in high estimated precision and highlighting the strength of the mixed-stock analysis in understanding questions of migratory connectivity with mtDNA.

The stable isotope and genetic data from this study compliment each other, strengthening conclusions in areas where uncertainty from just one technique would otherwise limit inferences. Stable isotope data from individual birds showed the same timing pattern as genetic data that grouped birds at weekly intervals, which supports the assignment of groups of birds to western breeding regions using genetic markers. Specifically, an influx of more depleted stable hydrogen isotope values coincided with a shift in mitochondrial groups at the middle of the migration season, suggesting that birds captured at the stopover site were migrating to more northern breeding areas. However, the influence of elevation and other geographic variables on stable hydrogen isotope values (Ingraham 1998) confounds the use of stable hydrogen isotopes alone to assign later migrants to particular breeding regions in western North America. For example, the

range of isotope values in Alaska (-80‰ to -140‰) also occur in southwest British Columbia, Washington, and interior Oregon and California. Thus, using only stable isotopes, I would only be able to postulate (rather than confidently determine) that later migrants were most likely migrating to Alaska. Fortunately, the lack of mitochondrial group 2 in migrants captured at the stopover site, common in the Sierra Nevada mountains of eastern California as well as eastern Oregon, reduced the uncertainty of assignment to the regions with overlapping isotope values, strongly suggesting that birds captured at the stopover site were migrating to breeding regions in Alaska and western Canadian provinces, not interior western regions. Compared with results drawn from stable isotopes alone, the genetic marker results allow stronger inferences about the timing of migration for specific populations in this study and the leapfrog migration pattern advanced previously by Paxton et al. (2007) at this stopover site.

Likewise, stable isotope data from migrating Wilson's Warblers highlight the need for more extensive sampling of genetic markers across Wilson's warbler's breeding range, especially in northwestern Canada and Alaska. Mitochondrial group 3 was found in low frequency across the breeding range sampled by Kimura et al. (2002) but was fairly common in the migratory stopover samples in the second half of the season, which suggests that there may be a large breeding population where this mtDNA group is common. Stable isotope values from migrants captured with mitochondrial group 3 were quite depleted, consistent with a northern boreal forest location, and indicate that more sampling in western Canadian provinces and Alaska may reveal the presence of this poorly known mitochondrial group.

The lack of mitochondrial group 2 in Wilson's Warblers captured at the stopover

site was somewhat surprising, given the prevalence of this mitochondrial group in breeding locations northwest of the stopover site, including the Sierra Nevada Mountains of eastern California as well as eastern Oregon. The Colorado River is a major flyway for western migratory birds (Rosenberg et al. 1991), and I would expect birds from these breeding regions to stop over in critical riparian habitat at our stopover site on the lower Colorado River. Yet results from this study suggest that Wilson's Warblers breeding in the Interior West use different migration routes than birds breeding on the coast; these routes are presumably east of Cibola NWR. Using stable hydrogen isotopes, Paxton et al. (2007) found strong connectivity of migration routes for warblers with different breeding populations using distinct migratory pathways across the southwestern United States from west to east. Warblers captured at western migration stations were dominated by populations breeding within coastal regions, whereas eastern migration stations had larger proportions of warblers breeding at more northern latitudes such as the western Canadian provinces and Alaska. However, there was not a continuous gradient of change in populations migrating across the southwestern migration routes. Thus, factors such as topography and habitat features likely play a role in shaping different migratory pathways for birds breeding in coastal and interior mountainous regions.

This is an exciting time in migratory connectivity research, given the technological advances that now allow us to link the movement of small landbirds throughout their annual cycle (Veen 2013). The results of this study show that by focusing on groups of birds (rather than individuals), we can use mtDNA variation to infer patterns of timing of migration for Wilson's Warblers at a finer scale of resolution than was previously thought feasible (Clegg et al. 2003). The approach used in the

present study has the potential to be applied to many species of birds where geographic structure has already been documented among populations using mtDNA (Newton 2003, Price 2008), potentially increasing our understanding of migratory connectivity for many species (e.g., Paxton et al. 2011). In addition, new modeling approaches offer much promise for integrating multiple markers (e.g., genetic and stable isotope markers) to infer patterns of migratory connectivity (Wunder 2010, Chabot et al. 2012, Rundel et al. 2013). For example, the Bayesian framework used by Rundel et al. (2013), which combines microsatellite and stable isotope markers for Wilson's warblers and Hermit Thrushes (*Catharus guttatus*), allows for more precise estimates of the breeding location of individuals captured across the wintering range. New approaches for understanding patterns of migratory connectivity of small landbirds are essential for determining the population dynamics of migratory species. Only through linking the phases of a migratory species' annual cycle and documenting seasonal interactions will we begin to develop a full understanding of the ecology of migratory species (Webster et al. 2002). The results of this study provide important insights into differences in the patterns of timing and migration routes for western breeding populations of Wilson's warblers, laying the foundation for more in-depth examination of seasonal interactions between migratory and stationary periods of the annual cycle.

APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL



The University of
Southern Mississippi

Institutional Animal Care
and Use Committee

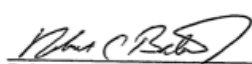
118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/upa/policies/animals

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION**

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.


PROTOCOL NUMBER: 09012601
PROJECT TITLE: **Migratory Connectivity and the En Route Migration Strategies of Migratory Birds**
PROPOSED PROJECT DATES: 03/15/09 to 03/14/11
PROJECT TYPE: **Renewal of a Previously Approved Project**
PRINCIPAL INVESTIGATOR(S): **Frank R. Moore, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **National Science Foundation**
IACUC COMMITTEE ACTION: **Designated Reviewer Approval**
PROTOCOL EXPIRATION DATE: **09/30/2011**


Robert C. Bateman, Jr., Ph.D.
IACUC Chair

2-2-09
Date

APPENDIX B

FEDERAL BANDING PERMIT



United States Department of the Interior
 U.S. GEOLOGICAL SURVEY
 PATUXENT WILDLIFE RESEARCH CENTER
 BIRD BANDING LABORATORY
 12100 BEECH FOREST ROAD STE-4037
 LAUREL, MD 20708-4037
 301-497-5790

Page 1 of 7

FEDERAL BIRD BANDING PERMIT

<p>Permittee: Personal DR FRANK R MOORE</p> <p>DEPT BIO SCI, UNIV SOUTHERN MS BOX 5018 HATTIESBURG, MS 39406 0001</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;">Permit Number:</td> <td style="width: 15%;">21221</td> <td style="width: 15%;">Action:</td> <td style="width: 15%;">Renew</td> <td style="width: 15%;">Action Date:</td> <td style="width: 15%;">04/28/10</td> <td style="width: 15%;">Issue Date:</td> <td style="width: 15%;">11/23/79</td> <td style="width: 15%;">Valid Until:</td> <td style="width: 15%;">07/31/13</td> </tr> <tr> <td colspan="10"> Signature of Issuing Official, Chief, Bird Banding Laboratory <i>F. Bruce Pesterfeld</i> </td> </tr> <tr> <td colspan="10"> Signature of Permittee </td> </tr> </table>	Permit Number:	21221	Action:	Renew	Action Date:	04/28/10	Issue Date:	11/23/79	Valid Until:	07/31/13	Signature of Issuing Official, Chief, Bird Banding Laboratory <i>F. Bruce Pesterfeld</i>										Signature of Permittee									
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Permittee agrees to band in accordance with the general conditions of this permit and with the specific authorization/s listed below:

Permittee is Authorized To Band:

All Species Except Waterfowl, Eagles or Endangered/Threatened Species Unless Specified Hummingbirds

In the States of:

AL * AK * AZ * CA * FL * LA * MA * MI * MS * NH * NY * RI * VT *

With Special Authorization to:

Use USGS Bands On Above Listed North American Migrants in * Honduras * Upon Host
 Country Approval
 Trap
 Use mist nets
 Band
 Take blood samples

**And Additionally Authorized to Use The Following
 Auxiliary Marking Authorization/s:**

Marker Type	Species	Colors	Locations	Comments
01A Plastic	SWAINSON'S THRUSH	Pink, Brown,	Baldwin, AL	
Colored Leg	GRAY-CHEEK THRUSH	Purple, Orange,	Mobile, AL	
Band	WOOD THRUSH	Black, White,	Cameron, LA	
	HOODED WARBLER	Green, Blue,	MS	
	KENTUCKY WARBLER	Yellow, Red		
	NO WATERTHRUSH			
	TENNESSEE WARBLER			
	BLUE-WING WARBLER			
	PROTHONOTARY WARB			
	RED-EYED VIREO			
01A Plastic	AMERICAN REDSTART	White, Black,	Mobile, AL	ALSO NAIL POLISH ON
Colored Leg	GRAY CATBIRD	Orange, Purple,	Baldwin, AL	FEDERAL BAND
Band	MAGNOLIA WARBLER	Brown, Red,		
	BLK-AND-WH WARBLER	Yellow, Blue,		
		Green, Mauve,		
		Pink		
89 Radio	CHUCK-WILL'S-WIDOW		Baldwin, AL	NTE 3% TOTAL BODY
Transmitter	SWAINSON'S THRUSH		Vernon, LA	WT; FREQ=216-217
	GRAY CATBIRD			MHZ; Glued attachment

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Signature of Issuing Official, Chief, Bird Banding Laboratory <i>Bruce Peterson</i>					
Signature of Permittee					

Permittee agrees to band in accordance with the general conditions of this permit and with the specific authorization/s listed below:

Marker Type	Species	Colors	Locations	Comments
89 Radio Transmitter	WOOD THRUSH OVENBIRD LA WATERTHRUSH		AL LA MS	NTE 3% TOTAL BODY WT; FREQ=216-217 MHZ; LEG LOOP ATTACHMENT
01A Plastic Colored Leg Band	OVENBIRD	Yellow, White, Pink	Hancock, MS	
89 Radio Transmitter	HOODED WARBLER		LA MS	NTE 5.0% TOTAL BODY WT; GLUE ATTACHMENT; 216-217 MHZ; EXPERIMENTAL OVERWEIGHT AUTHORIZATION; REPORT ALL ADVERSE EFFECTS
01A Plastic Colored Leg Band	GRAY CATBIRD COM YELLOWTHROAT WHITE-EYED VIREO	White, Green, Blue, Yellow, Red, Orange, Black	Jackson, MS	
89 Radio Transmitter	WILSON'S WARBLER		Yuma, AZ	NTE 5.0% TOTAL BODY WT; GLUE ATTACHMENT; 216-217 MHZ; EXPERIMENTAL OVERWEIGHT AUTHORIZATION; REPORT ALL ADVERSE EFFECTS
89 Radio Transmitter	RED-EYED VIREO		Cameron, LA Vernon, LA	NTE 3% total body weight, Glue attachment, 216 to 217 MHz
01A Plastic Colored Leg Band	WILSON'S WARBLER	Black, Blue, Brown, Green, Orange, Pink, Purple, Red, White, Yellow	Yuma, AZ	
89 Radio Transmitter	RED-EYED VIREO GRAY CATBIRD SWAINSON'S THRUSH		Baldwin, AL	NTE 5% total body weight, glue attachment, 164 to 166 MHz. Report any negative impacts to BBL.

Permittee: Personal DR FRANK R MOORE DEPT BIO SCI, UNIV SOUTHERN MS BOX 5018 HATTIESBURG, MS 39406 0001	Permit Number: 21221	Action: Renew	Action Date: 04/28/10	Issue Date: 11/23/79	Valid Until: 07/31/13
Signature of Issuing Official, Chief, Bird Banding Laboratory <i>Bruce Peterson</i>					
Signature of Permittee					

Permittee agrees to band in accordance with the general conditions of this permit and with the specific authorization/s listed below:

- 21221 - M** MS EMILY COHEN DEPT BIO SCI, UNIV SOUTHERN MS BOX 5018
HATTIESBURG, MS 39406 5018
- Is Authorized To Band:**
- Passerines and Near-passerines
- In the States Of:**
- LA *
- With Special Authorization to:**
- Trap
Use mist nets
Band
Auxiliary mark
- 21221 - N** MS KRISTINA PAXTON DEPT BIO SCI, UNIV SOUTHERN MS 118 COLLEGE
DRIVE HATTIESBURG, MS 39406 0001
- Is Authorized To Band:**
- All Species Except Waterfowl, Eagles or Endangered/Threatened Species Unless Specified
- In the States Of:**
- AL * AK * AZ * CA * LA * MS *
- With Special Authorization to:**
- Trap
Use mist nets
Band
Auxiliary mark
- 21221 - O** MS JACLYN SMOLINSKY DEPT BIO SCI, UNIV SOUTHERN MS BOX 5018
HATTIESBURG, MS 39406 0001
- Is Authorized To Band:**
- Passerines and Near-passerines
- In the States Of:**
- AL * LA * MS *
- With Special Authorization to:**
- Trap
Use mist nets
Band
Auxiliary mark

FEDERAL BIRD BANDING PERMIT

Under the provisions of Regulations issued under the Migratory Bird Treaty Act of July 3, 1918 (40 Stat. 755) as amended, or the Bald Eagle Act of June 8, 1940 (54 Stat. 250) as amended, the person named hereon is authorized to capture, for scientific banding or marking purposes, those migratory birds described hereon and to salvage birds accidentally killed during normal banding activities.

This permit is subject to the terms, exceptions and restrictions expressed herein and is further subject to any applicable Territorial, State, Tribal or Federal Regulations.

This permit is invalid unless accompanied by any required State permits or licenses.

GENERAL CONDITIONS

1. The Permittee is not authorized to capture or possess migratory birds for any reason other than banding, marking or salvage of banding mortalities for scientific purposes. **NOR IS THE PERMITTEE ALLOWED TO HOLD MIGRATORY BIRDS FOR A PERIOD OF MORE THAN 24 HOURS** except that the Permittee may possess, for the purpose of donating to a public, scientific or educational institution, birds which die as a result of normal banding activities. Live birds shall be released as soon as practical after capture.
2. The Permittee shall keep RECORDS accounting for the use of all bands received. Periodic RECORDS COVERING THE USE OF THESE BANDS shall be submitted to the Bird Banding Laboratory in accordance with the instructions received there from. Failure to provide data in accordance with the instructions received from the Bird Banding Laboratory is sufficient justification for the revocation of this permit. The Permittee shall keep records of disposition of salvaged banding mortalities for a period of five years and shall be reported to the Bird Banding Laboratory upon request.
3. The holder of this permit shall not sell, exchange, or transfer bands to unauthorized banders or to the general public. All transfers to authorized banders must be communicated to the Bird Banding Laboratory prior to the transfer of bands. Any unused bands remaining when this permit is voluntarily returned, revoked, or expired must be returned to the Bird Banding Laboratory.
4. The Permittee shall, at all reasonable hours, allow any authorized representative of the U. S. Geological Survey or the U.S. Fish and Wildlife Service to ENTER and INSPECT the premises where operations authorized by this permit are being conducted and shall allow such representative to inspect the records relating to such operations.
5. This permit may be SUSPENDED or REVOKED by the Director of the U.S. Geological Survey or authorized representative, if the Permittee violates any of the provisions in the regulations under which this permit is issued or if the Permittee fails to render promptly any reports required. This permit is, at all times, subject to suspension or revocation at the discretion of the Director or representative.
6. This permit is not transferable and must be in possession of the Permittee when exercising the authorizations granted herein.
7. All traps, nets or other capture devices shall bear a TAG or LABEL showing the name, address and permit number of the Permittee; alternatively the trapping area shall be adequately marked with POSTERS provided by the Bird Banding Laboratory. The Permittee's name, address and permit number shall be legibly displayed on such posters.
8. This permit DOES NOT authorize the capture of any birds on any property, public or private without the CONSENT OF THE OWNER OR CUSTODIAN THEREOF.
9. All Banding under this permit is in accordance with the principles, spirit, and intent of the Animal Welfare Act of 1970 and the most recent revision of The Ornithological Council's Guidelines in the Use of Wild Birds in Research.
10. Unless specifically noted on the reverse, the following ARE NOT AUTHORIZED:
 - a. The taking of blood or feather sampling from any bird.
 - b. The use of ANY BAND, clip, paint, dye, signal-sending device or any marking device other than the official numbered leg bands issued by the Bird Banding Laboratory.
 - c. The use of MIST NETS or other nets for the capturing of birds.
 - d. The use of TRANQUILIZING DRUGS OR OTHER CHEMICALS for the purpose of capturing birds.
 - e. Trapping or disturbing the nests or nestlings, for the purpose of banding or marking, of species designated by the Secretary of Interior as "ENDANGERED" or "THREATENED."
 - f. The handling of any PREVIOUSLY Banded BIRD in any manner which may bias data on file in the Bird Banding Laboratory which pertain to that bird or which may alter that bird's survival potential, behavior or other normal characteristics. This specifically includes adding markers to or removing markers from previously banded birds.

Form 9-475
(July 2007)

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